Identifying Potential biomarkers of insulin sensitivity and insulin resistance in obese patients

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Chapter 1: Introduction to the thesis

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Introduction to the thesis

Obesity is a major risk factor for cardiovascular disease, hypertension, cancer and type 2 diabetes mellitus (T2DM) [1]. A subset of obese individuals, known as the obese insulin sensitive (OIS) or metabolically healthy obese (MHO), exhibit less comorbidities compared to their obese insulin resistant (OIR) counterparts [2]. The protected OIS individuals maintain insulin sensitivity [2, 3] and exhibit lower systemic levels of lipids and inflammatory markers compared to OIR individuals [4, 5]. Studies have also suggested that OIS individuals show less markers of oxidative stress [6, 7]. The underlying inflammatory and oxidative stress mediators could be induced by different genetic and environmental factors [8]. While the genetic component remains largely undetermined, the environmental effects of specific pollutants and medications were previously established [9]. However, the molecular pathways underlying the protective mechanisms in OIS remain unknown [10]. **In this thesis,** we describe an OMICS approach to identify these pathways in the blood and adipose tissues of OIS, OIR and T2DM individuals. The overall aim was to understand the underlying protective mechanisms in those at lower risk (OIS individuals), which could help in designing novel diagnostic and therapeutic strategies targeting those at higher risk of disease (OIR and T2DM individuals).

Obesity is associated with the expansion of adipose tissues through hypertrophy of mature adipocytes and the differentiation of local preadipocytes in a process known as adipogenesis to store excess

triacylglycerols (TAGs). Impairment of adipogenesis leads to ectopic fat deposition in skeletal muscles, liver, and kidneys, triggering insulin resistance in these tissues and increased risk of T2DM. Many factors contribute to impaired adipogenesis. **In chapter 2,** we review molecular mediators of impaired adipogenesis including inflammatory markers, oxidative stress, fatty acid signaling and environmental pollutants [12].

In chapter 3, we investigate the metabolic pathways of obesity-associated insulin resistance of blood samples from lean controls, OIS, OIR and T2DM obese individuals. In this chapter, we describe our data from 107 subjects who underwent untargeted metabolomics of serum samples using the Metabolon platform [13]. Thirty-two subjects were lean controls whilst 75 subjects were obese including 20 OIS, 41 OIR, and 14 T2DM individuals. Our data showed that various phospholipid metabolites were significantly altered among studied groups. Additionally, our data confirmed changes in known metabolic markers of liver disease, vascular disease and T2DM. In this chapter we discussed the roles of the identified metabolites in increasing risk of insulin resistance and associated comorbidities and highlighted the importance of future functional validation of these metabolites.

Since lipid intermediates were shown to play a role in the development of obesity-associated insulin resistance, **chapter4,** describes lipidomics approach used to investigate differences between subcutaneous (SC) and omental (OM) adipose tissues. These two fat depots have direct roles in the functional storage of lipid intermediates produced during triacylglycerols (TAGs) synthesis and lipolysis. These

intermediates interfere with the intracellular insulin signaling pathway in adipocytes and development of insulin resistance. In this chapter we compared TAG species and their fatty acid composition in adipose tissues from OIS, OIR and T2DM obese individuals [11]. Human SC and OM adipose tissues were obtained from 64 clinically characterized obese individuals during weight reduction surgery. TAGs were extracted from the adipose tissues using the Bligh & Dyer method, then were subjected to non-aqueous reverse phase ultra-high performance liquid chromatography and full scan mass spectrometry acquisition and data dependent MS/MS on LTQ dual cell linear ion trap. TAGs and their fatty acid contents were identified and compared among OIS, OIR and T2DM individuals and their levels were correlated with metabolic traits of participants and the adipogenic potential of preadipocyte cultures established from their adipose tissues. Our data showed that adipose tissues from OIR and T2DM individuals exhibit TAG-specific signatures that may contribute to their increased risk compared to their OIS counterparts. The chapter discussed the potential functional relevance of the identified TAG species and highlighted the importance of future functional experiments.

Polybrominated diphenyl ethers (PBDEs), a widely utilized class of flame retardants in various commercial products, represent a prominent source of environmental contaminants. PBDEs tend to accumulate in adipose tissue, potentially altering the function of this endocrine organ and increasing risk of insulin resistance. **Chapter 5**, summarizes profiling of levels of 28 PBDE congeners assessed in SC and OM tissues from 34 obese Qatari individuals (11 OIS and 23 OIR) using gas

chromatography (Trace GC Ultra) coupled to a TSQ Quantum triple Quadrupole mass spectrometer. Correlations of identified PBDEs and mediators of metabolic disease were established and the effects of PBDEs treatment on insulin signaling in primary OM preadipocytes were determined. Our data showed that accumulation of specific species of PBDEs in human adipose tissues was indeed associated with insulin resistance in obese individuals. The chapter also discussed the potential role of these environmental pollutants and highlighted the importance of future investigation of their functional role in the pathology of insulin resistance. [9]

Chapters 6,7 and **8** provide a comprehensive summary of the results and conclusions along with plans for future work related to functional investigation of the identified targets and the molecular pathways underling increased risk of insulin resistance in lean individuals.

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Chapter 2: Mediators of Impaired Adipogenesis in Obesity-Associated Insulin Resistance and T2DM

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Abstract

Obesity has become a global health issue due to its high prevalence and associated comorbidities including insulin resistance (IR) and type 2 diabetes mellitus (T2DM). Obesity is associated with the expansion of adipose tissues through hyper- trophy of mature adipocytes and differentiation of local preadipocytes in a process known as adipogenesis to store excess triacylglycerols (TAGs). Impairment of adipogenesis leads to ectopic fat deposition in skeletal muscles, liver, and kidneys, triggering IR in these tissues and increased risk of T2DM. Many factors contribute to impaired adipogenesis including obesity-associated mild chronic inflammation, oxidative stress, and fatty acid signaling. This review summarizes recent literature covering mediators of impaired adipogenesis and underlying molecular pathways.

Keywords: adipogenesis, mediators, inflammation, oxidative stress, fatty acids

1. Obesity-associated metabolic disease

Rapidly changing lifestyle, accompanied by consumption of excess energy in the form of a calorie-rich high-fat diet, lower voluntary activity, and increased exposure to environmental pollutants, have led to an exponential rise in noncommunicable metabolic diseases [1]. A key component of chronic metabolic diseases is obesity that has become a global health problem associated with a range of comorbidities including insulin resistance and type 2 T2DM [2], coronary artery disease (CAD) [3], nonalcoholic fatty liver [4], cancers [5], and elevated risk of premature death [6, 7].

Adipose tissue is an endocrine organ that responds to obesity by secreting elevated quantities of free fatty acids, adipokines, and proinflammatory cytokines, triggering IR and risk of T2DM [8]. Obesity is also characterized by increased adiposity mediated by enlarged size of mature adipocytes (hypertrophy) and elevated number of newly recruited adipocytes (hyperplasia) [9–12]. Adipose tissue dysfunction is characterized by adipocyte hypertrophy, mild chronic inflammation, and oxidative stress, causing reduced ability to generate new adipocytes from the undifferentiated precursors (preadipocytes). The impaired adipogenesis increases risk of IR and T2DM by triggering ectopic fat deposition in non-adipose tissues and proinflammatory environment characterized by impaired secretion of various adipose-derived adipokines [13].

Obesity also represents an imbalance between the primary site of storing energy (the white fat) and the site that is specialized in energy expenditure (the brown fat) [14]. White adipocytes store fat in the form of triacylglycerols as a single fat lipid droplet that gets readily hydrolyzed by lipases when energy is needed. The resulting fatty acids are mobilized to other tissues to undergo fatty acid oxidation as a source of energy [15]. The imbalance between lipolysis and lipogenesis plays a crucial role in progression of metabolic disease including T2DM and non-alcoholic fatty liver disease [16]. The brown fat, on the other hand, uses the energy derived from fatty acid oxidation for heat generation [17].

Adipocyte hypertrophy is associated with increased uptake of excess TAGs, which triggers fat accumulation within the larger subcutaneous

adipose tissue (SAT) [18–20]. SAT therefore plays a buffering role as it prohibits progression of obesity-associated pathologies [21]. However, the buffering capacity becomes limited as impairment of SAT expansion causes IR [22–24] as the excess fat are deposited in the visceral adipose tissue (VAT) as well as ectopically in the skeletal muscle, liver, kidney, and heart tissues [25]. This is augmented by the infiltration of macrophages and activation of the innate immune cells [26], which triggers hyper- insulinemia that inhibits lipolysis and activates lipoprotein lipase (LPL). This causes further hyperinsulinemia, hypertriglyceridemia, increased IR in these tissues [27], and risk of T2DM [28].

Although obesity is generally associated with these comorbidities, some obese individuals seem to be protected as they maintain insulin sensitivity (IS) and show lower hypertension and proatherogenic and inflammatory profiles than their equally obese pathogenic counterparts [29–32]. Investigating the underlying causes for this protective phenotype could potentially help obesity-associated pathogenicity. Although still unknown, various potential mechanisms were proposed to contribute to metabolically healthy obese (MHO) phenotype. These include lower visceral and ectopic fat deposition than subcutaneous fat accumulation due to efficient SAT adipogenesis, reduced inflammatory component in the adipose tissue, healthy levels of secreted adipokines, and more active lifestyle [33]. A genetic component was also suggested to interact with various environmental factors, although not yet determined [34]. Interestingly, lean diabetics also exhibit larger adipocytes than healthy individuals, perhaps due to impaired differentiation of preadipocytes but not a result of different frequencies of stromal vascular cells,

lipolysis, or levels of inflammatory mediators [35]. Current therapeutic strategies focus on treating obesity-associated diseases instead of preventing the underlying mechanisms. Therefore, understanding the molecular mediators underlying the protective phenotype in MHO individuals could provide critical information to help individuals suffering from pathological obesity (PO). In this review, we aimed to understand the role of adipogenesis in obesity-associated IR and T2DM by screening 2317 articles investigating adipogenesis and mediators of impaired adipogenesis in PubMed with the aid of Rayyne, a systematic review web application [36].

2. The role of adipogenesis in obesity-associated IR and T2DM

The adipose tissue is a dynamic part of the endocrine system that plays a crucial role in maintaining energy balance and nutritional homeostasis [37]. Mature adipocytes constitute the most abundant distinctive cell type in the adipose tissue, occupying 90% of its volume [38]. Other components include leukocytes, macrophages, fibroblasts, endothelial cells, and preadipocytes, which constitute the stromal vascular cells (4–6 million cells per gram of adipose tissue, half of which are immune cells) [39].

Obesity-induced adipocyte hypertrophy is associated with impaired recruitment and differentiation of preadipocytes. Despite their abundance, preadipocytes fail to undergo terminal differentiation into mature adipocytes via the activation of the canonical Wnt signaling [40]. Preadipocytes are produced by mesenchymal stem cells (MSCs) under

the influence of different signaling molecules. The mature adipocytes secrete BMP4 that triggers preadipocyte differentiation by inducing the separation of Wnt1 inducible-signaling pathway protein 2 (WISP2) and zinc finger protein 423 (ZNF423), allowing ZNF423 to translocate into the nucleus and activate peroxisome proliferator-activated receptors (PPARγ) and downstream cascade including CCAAT/enhancer-binding proteins β (C/ EBPβ), δ, and α [41, 42] (**Figure 1**). BMP4 also plays an anti-inflammatory role by reducing tumor necrosis factor-α (TNF-α)-mediated proinflammatory cytokine induction in human adipocytes. Therefore, BMP4 plays a protective role against IR and T2DM [43]. Subsequently, PPARγ and C/EBPα activate preadipocyte differentiation and the expression of mature makers such as adiponectin, fatty acid-binding protein 4 (FABP4), glucose transporter type 4 (GLUT4), and LPL. The activation of PPARγ, therefore, maintains IS and exhibits an anti-inflammatory function, whereas IR causes impaired adipogenesis and increased risk of T2DM [44, 45].

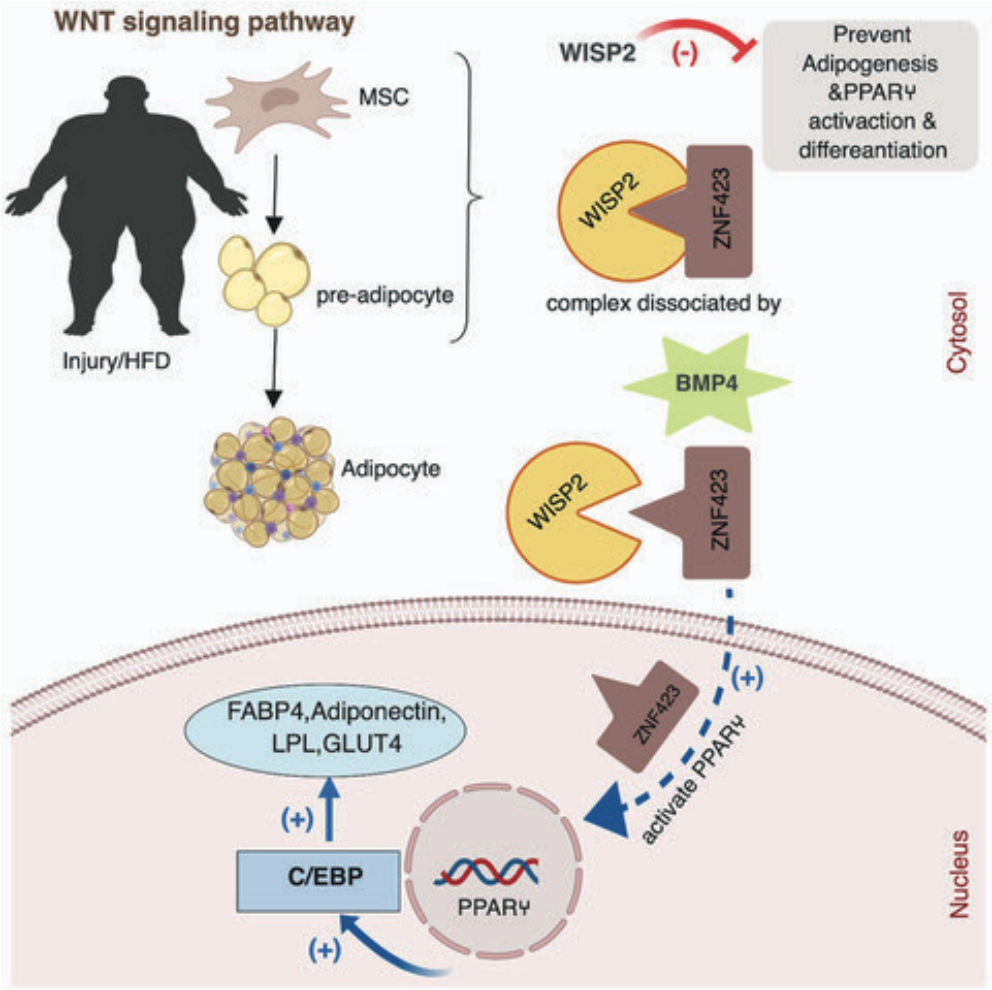

Figure 1. Schematic representation of the role of Wnt signaling in adipogenesis.

Insulin and downstream Akt signaling also play important roles as modulators of adipose tissue growth and adipogenesis as insulin activates glucose and free fatty acid uptake, inhibits lipolysis, and de novo fatty acid synthesis in adipocytes, and induces adipogenesis [46]. The transcription factor nuclear factor kappa-light- chain-enhancer of activated B cells (NF-κB) has been shown to induce energy expenditure and reduce adipose tissue growth, leading to prevention of dietary obesity and lowering adipogenesis, inflammation, and IR [47]. The inhibition of inhibitor of nuclear factor kappa-B kinase subunit β (IKKβ) in mice lowers high- fat diet-induced adipogenesis and inflammation and protects from diet-induced obesity and IR [48]. MicroRNAs (miRNAs) have been also shown to play an important role in adipogenesis, IR, and inflammation as previously reviewed [49]. Tonicity-responsive enhancer-binding protein (TonEBP), a key transcription factor involved in cellular adaptation to hypertonic stress, has been suggested to influence macrophage activity, adipogenesis, and IS by inhibiting the epigenetic transition of PPARγ2 [50]. Protectin DX (PDX), a ω-3 fatty acid-derived proresolution mediator, was reported to reduce inflammation and IR via an AMPK-dependent pathway and suppress adipogenesis and lipid accumulation during 3T3-L1 differentiation [51].

We have recently shown that higher adipogenic capacity of preadipocytes iso- lated from SAT and VAT from MHO individuals than PO counterparts may be one of the underlying mechanisms for MHO protection due to a greater ability to store TAGs in the SAT depot. This process was shown to be influenced by inflammatory mediators, oxidative stress, and fatty acid signaling [45, 52–55].

3. Mediators of impaired adipogenesis in IR and T2DM

3.1 Inflammatory mediators

3.1.1 Impaired adipogenesis in response to proinflammatory signals

Obesity-associated comorbidities are mediated by chronic mild inflammation (Figure 2). Lipid-laden adipocytes produce increased levels of cytokines such as Interleukin 6 (IL-6), IL-β, TNF-α, monocyte chemoattractant protein-1 (MCP-1), and IL-8 [10, 56, 57] which can inhibit preadipocyte differentiation [21, 45]. The impaired adipogenesis is associated with stress of the endoplasmic reticulum (ER) and elevated expression of unfolded protein response (UPR), both can exacerbate the proinflammatory phenotype of preadipocytes and adipocytes [58]. The effect of proinflammatory phenotype varies among various fat depots. VAT is a more inflammatory tissue than SAT as it secretes higher levels of proinflammatory cytokines. Macrophage infiltration into adipose tissue is regulated through serum resistin and leptin in obese individuals with early metabolic dysfunction [59].

The presence of macrophages in VAT contributes significantly to this phonotype. The presence of macrophages in human SAT, on the other hand, is causally related to impaired preadipocyte differentiation, which in turn is associated with systemic IR [60, 61]. Adipocyte differentiation, therefore, was shown to be significantly lower in VAT than SAT. Macrophage depletion can reduce inflammatory cytokines and trigger adiponectin secretion from both SAT and VAT adipocytes, leading to the induction of preadipocyte differentiation in SAT, but not VAT.

Additionally, a negative correlation between SAT adipogenesis, but not VAT, and systemic IR was observed [62]. Chronic systemic inflammation is also associated with elevated lipolysis in white adipose tissue and lipogenesis in non-adipose tissues, causing ectopic fat deposition in non-adipose tissues and imbalance in free fatty acid homeostasis and increased risk of IR [63].

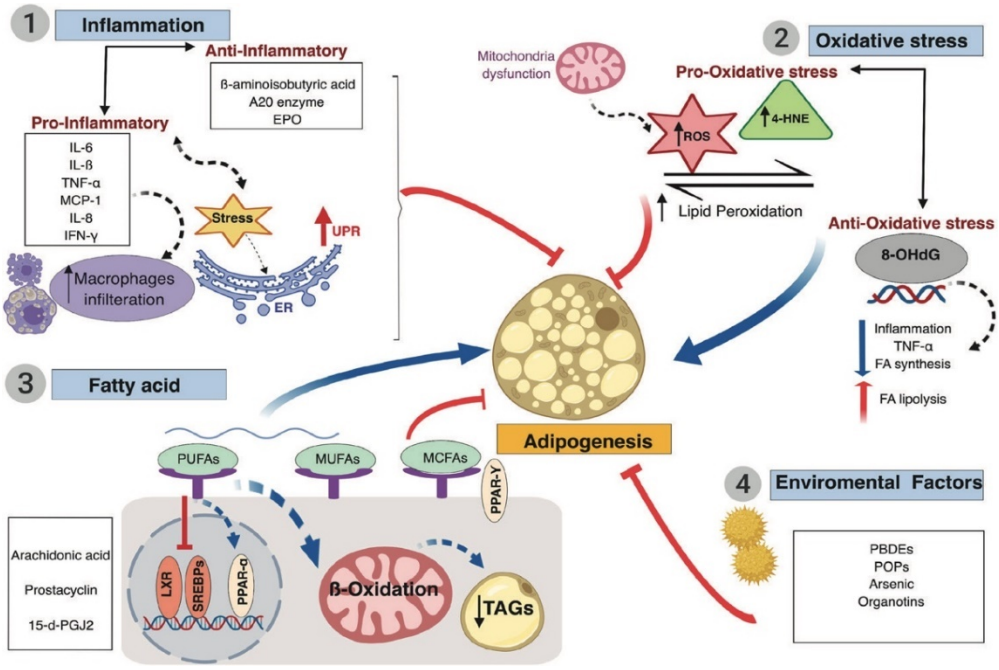

Figure 2. Mediators of impaired adipogenesis in IR and T2DM. Most proinflammatory cytokines as well as some anti-inflammatory mediators can impair adipogenesis (1). Similarly, various mediators of oxidative stress can impact adipogenesis both positively and negatively depending on their structure (2). Fatty acid signaling plays a key role in adipogenesis but at various degrees depending on the composition of the fatty acids (3). Finally, various environmental factors can impact adipogenesis mostly negatively (4).

Among the proinflammatory cytokines, IL-6 is produced by adipocytes, activated leukocytes, and endothelial cells [64] in obesity [65–68]. IL-6 shows a synergistic effect with other mediators of metabolic disease, collectively contributing to the progression of other obesity-associated comorbidities such as CAD and T2DM [64, 69]. IL-6 impairs the LPL function leading to increased levels of circulating fat [69, 70]. Moreover, obesity-associated increase in IL-6 is linked to reduced insulin-triggered glucose uptake [60, 61]. Previous reports have indicated that insulin treatment improves the glucose transport activity of adipocytes in T2DM [21] and lowers IL-6 and TNF-α levels [53]. Although the precise mechanisms of IL-6-associated IR is not well characterized, human adipocytes from IR individuals were shown to exhibit significantly higher IL-6 expression levels [45]. IL-6 impairs insulin action by inhibiting expression of insulin receptor, insulin receptor substrate-1 (IRS-1), and GLUT4 in human preadipocytes as well as 3T3-L1 adipocytes [45, 71]. Furthermore, IL-6 was shown to reduce IS through decrease in adiponectin expression and secretion [72] and via impairment of insulin signaling in hepatocytes [73].

Various other cytokines have been shown to impact adipogenesis [74]. The proinflammatory cytokines IL-1 β, TNF-α, and MCP1 can also influence the hyper- plastic expansion of adipose tissue and impair adipogenesis [59]. IL-1β triggers a proinflammatory response in human adipose tissues, particularly in VAT depot. IL-1β also inhibits insulin signal transduction, leading to impaired IS in adipose tissue [75]. IL-1β and cyclooxygenase-2 (COX-2) play a detrimental role in adipose tissue dysfunction in obesity [76]. With obesity, levels of MCP-1 and TNF-

α increase in VAT before macrophage infiltration, suggesting a highly proinflammatory phenotype of the visceral depot prior to infiltration of immune cells and macro- phage phenotype switch [77]. Unlike IL-6, IL-1 β, and TNF-α, MCP-1 and MCP- 1-induced protein (MCPIP) were shown to induce adipogenesis. Treatment of reactive oxygen species (ROS) inhibitor, apocynin, reduced the MCPIP-triggered adipogenesis [78]. Other cytokines involved in adipogenesis include interferon-γ (IFN-γ), a central mediator of macrophage function. Compared to obese wild-type control animals, obese IFN-γ knockouts exhibit better IS, smaller adipocyte size, and lower cytokine expression [79].

3.1.2 Impaired adipogenesis in response to anti-inflammatory signals

Contrary to the notion that inflammation plays a negative role in metabolism, some studies suggest that proinflammatory signals in the adipocytes are actually needed for functional adipose tissue homeostasis (**Figure 2**). Indeed, adipose tissue inflammation was shown in various animal models of adipose tissue-specific
reduction of proinflammatory potential to be required as an adaptive response, allowing proper storage of excess fat and filtering of gut-derived endotoxins [80]. Additionally, various molecules with anti-inflammatory properties were shown to influence adipogenesis and risk of IR. Myokines, for example, secreted by skeletal muscle cells during exercise such as β-aminoisobutyric acid, can impair adipogenesis via activating AMPK signaling pathway and reducing levels of proinflammatory cytokines such as TNF-α [81]. Another example is the ubiquitin-

editing enzyme A20 that impairs IL-6 secretion from adipocytes, leading to modulation of differentiation of MSCs [82]. The overexpression of A20 was also shown to reduce lipo- genesis and adipogenesis via lowering levels of sterol regulatory element binding protein-1c (SREBP-1c) and aP2, causing lower fat accumulation in differentiated 3T3-L1 cells [83]. A third example is the nonerythropoietic EPO-derived peptide that plays an anti-inflammatory and anti-adipogenic roles in high-fat die mice with IR [84]. On the other hand, other anti-inflammatory molecules could rescue impaired adipogenesis. Glucose-dependent insulinotropic polypeptide (GIP), for example, is a potent activator of adipogenesis through modulation of inflammation in adipose tissue [85]. Additionally, the expression of neuronatin (Nnat), a proteolipid involved in neuronal development, in response to inflammation and dietary excess, has been suggested to play an important role in adipogenesis through lowering oxidative stress and inflammation [86].

3.2 Oxidative stress

Obesity leads to the accumulation of ROS, the hallmark of oxidative stress, in the adipose tissue causing impaired adipogenesis and increased risk of IR and T2DM. The balance between ROS generation and activation of endogenous anti- oxidants is crucial for cells undergoing adipogenesis [87] (Figure 2). The oxidative damage and changes in the expression of antioxidant enzymes with age are similar between SAT and VAT. However, preadipocytes from SAT are significantly more resistant than VAT-derived cells to cell death caused by oxidative stress [88]. Interestingly, within SAT and VAT depots, preadipocytes from

insulin-sensitive obese subjects were more prone to oxidative damage than preadipocytes from equally obese insulin-resistant individuals [52, 53]. The depletion of ROS from adipose tissue in mice models of oxidative stress was associated with increased adipose tissue mass, lower ectopic fat deposition, and enhanced IS. Similarly, ROS accumulation limited the expansion of adipose tissue, leading to elevated ectopic fat accumulation and increased risk of IR [89]. Elevated ROS within the adipose tissue triggers lipid peroxidation [45] and accumulation of reactive aldehydes including the bioactive lipid peroxidation product 4-hydroxynonenal (4-HNE) [90]. Elevated 4-HNE causes damage of cell structure and function through the formation of the stable adducts 4-hydroxyalkenals with proteins, phospholipids, and DNA [91, 92]. Increased 4-HNE levels have been associated with impaired adipogenesis and IR [53, 93–96]. Another marker of oxidative damage is 8-hydroxy-2-deoxyguanosine (8-OHdG) which was recently shown to exert anti-inflammatory effects, by reducing TNF-α- induced IR in vitro. It was also shown to reduce adipose tissue mass in vivo through activation of adipose triglyceride lipase and lowering the expression of fatty acid synthase [97]. Levels of cholesterol oxidation-derived oxysterols increase in adipose tissues of T2DM patients and act as inhibitors of adipogenesis through activation of Wnt pathway [98]. Heme oxygenase (HO), a major cytoprotective enzyme, functions upstream of Wnt signaling and lowers lipogenesis and adipogenesis, decreasing lipid accumulation and levels of proinflammatory cytokines [99].

Conversely, ROS was also shown to enhance adipogenesis by lowering sirtuin 1 (Sirt1) expression [100, 101]. Heme-induced oxidative stress was shown to inhibit Sirt1, leading to increased adipogenesis [102]. The

expression of deleted in bladder cancer protein 1 (DBC1), another inhibitor of the Sirt1, is reduced with obesity, leading to lower adipogenesis and VAT dysfunction [103]. Sirt3 plays a crucial role in mitochondrial function. Silencing of Sirt3 can cause adipocyte dysfunction which impairs adipogenesis and causes IR [104]. Nonselenocysteine-containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) is a sensor of oxidative stress. Lack of NPGPx causes elevation in ROS and promotion of adipogenesis through ROS-dependent dimerization of protein kinase A regulatory subunits and activation of C/EBPβ [105]. Additional evidence suggesting ROS involvement in promotion of adipogenesis comes from antioxidant supplementation experiments where lower levels of ROS resulting from antioxidants contribute to adipose tissue dysfunction and IR [106]. Indeed, antioxidant supplementation exhibited a negative impact when used before induction of oxidative stress as a result of lowering physiological ROS levels because ROS plays a role as second messengers in adipogenesis, lipid metabolism, and insulin signaling [107]. For example, the supplementation with N-acetylcysteine, a known antioxidant and precursor of glutathione, was shown to reduce fat deposition during adipogenic differentiation of mouse fibroblasts [108]. Activation of beta-3 adrenergic receptor (β3-AR) enhances ROS accumulation in cultured adipocytes. Antioxidants enhance β3-AR- triggered mitochondrial ROS production, suggesting that chronic supplementation of antioxidants could indeed generate an elevation in oxidative stress associated with mitochondrial dysfunction in adipocyte [109]. On the other hand, glutathione depletion was shown to inhibit adipogenesis as the result of lowering cell proliferation during

the initial mitotic clonal expansion of the adipocyte differentiation process [110].

3.3 Fatty acid signaling

The main role of adipocytes is TAG storage. Although TAGs do not function as signaling molecules per se, the lipid intermediates generated during lipogenesis and lipolysis influence intracellular insulin signaling and participate in progression of IR. These include free fatty acids, diacylglycerols (DAGs), and ceramides [111].

Lipolysis-driven efflux of fatty acids triggers TAG synthesis and causes stress of the ER and activation of June kinase pathway in the adipose tissues 112, 113]. This leads to an elevation in the levels of both DAGs and ceramides and progression of IR in adipocytes [114]. Ceramides were shown to influence lipid-mediated IR in muscles. Delta 4-desaturase, sphingolipid 1 (DEGS1) is a desaturase that mediates ceramide biosynthetic pathway. Ablation of DEGS1 in preadipocytes prevented adipogenesis and decreased lipid accumulation [115]. There are essential enzymes responsible for TAG hydrolysis including hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and monoglyceride lipase (MGL) [116].
ATGL regulates lipolysis by transcription factor specificity protein 1 (Sp1). Insulin-mediated transcription of Sp1 is critical for this regulation. In mature adipocytes, PPARγ reverses transcriptional repression by Sp1 at the ATGL promoter, leading to stimulation of ATGL mRNA expression. During obesity and IR, the transcription of ATGL becomes

downregulated. The extent of the downregulation depends on interactions between Sp1 and PPARγ [117].

A number of factors influence the function of fatty acids in regulating adipo- genesis. The number of carbons and the position and number of double bounds are crucial determinants of properties of the fatty acids. Changes in fatty acids including elongation, desaturation, β-oxidation, peroxidation, and incorporation into phospo and complex lipids can play an essential role in their metabolic function. Fatty acids and their metabolites can control protein expression involved in lipid and energy metabolism by influencing gene transcription\n, mRNA processing, and posttranslational modifications [118–121]. Most fatty acids activate all three members of the PPAR family [122–125]. Polyunsaturated fatty acids (PUFAs), except for erucic acid, are more potent stimulators of PPARγ than monounsaturated fatty acids (MUFAs) and saturated fatty acids [122–126] (Figure 2). The optimal binding affinity is reached with 16–20 carbon-containing compounds. DHA too was shown to stimulate PPARs [124]. Various studies have reported the beneficial effects of PUFAs on lipid-related human disorders [127–131], which largely depend on the structure of the fatty acids and their metabolic properties. PUFAs can inhibit lipo- genic gene transcription by downregulating the expression SREBPs [132–135] and act as antagonists of liver X receptors (LXR) [136, 137] and as agonists for PPARs [122–124, 138, 139]. PUFAs, but not saturated or MUFAs, inhibit lipogenic genes by downregulating SREBP-1c. PPAR alpha plays an important role in metabolic adapta- tion to fasting by enhancing mitochondrial and peroxisomal fatty acid oxidation and ketogenesis [140]. Dietary PUFAs were also

shown to stimulate expression of PPARα target genes, induce β-oxidation, and lower plasma TAGs [141–149]. Fatty acids can also play a role as modulators of kinase signaling pathways [150–155].

Arachidonic acid (AA), a polyunsaturated omega-6 fatty acid, is the major PUFA that has been implicated in the regulation of adipogenesis. Short exposure of 3T3-L1 mouse preadipocytes to AA triggers adipocyte differentiation, associated with increase in (FABP4/aP2). Calcium, protein kinase C, and ERK play critical role in this pathway through which AA induces the expression of adipocyte protein 2 (aP2) [156]. AA binds to PPAR-γ2 to stimulate GLUT4 expression in HepG2 cell line, exhibiting an alternative insulin-independent activation of GLUT4 [157]. AA cascade is then controlled by cyclooxygenases enzymes, lipoxygenases, and P450 epoxygenases. When AA is generated from plasma membrane via phospholipases and then metabolized by prostaglandin G/H synthase, different prostaglandins are produced, causing opposing effects on adipocyte differentiation. The proadipogenic effect of AA is mediated by prostaglandin product (prostacyclin) and is thus cyclooxygenase dependent [158–160]. Among prostaglandin classes, 15-deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2) was shown to be proadipogenic [161, 162]. On the other hand, prostaglandin F2α (PGF2α) was shown to exert anti-adipogenic effects in primary preadipocytes [163–165], 1246 cells [164], and 3T3-L1 cells [166–168]. The anti-adipogenic effect of PGF2α is mediated through prostaglandin F receptor-mediated elevation in intracellular calcium and DNA synthesis [168] and activation of MAPK, causing reduction in PPARγ phosphorylation [169]. The role of prostaglandin E2 (PGE2), the third main prostaglandin, in

adipogenesis is controversial as PGE2 exhibits antilipolytic effect in mature adipocytes but shows no effect on preadipocytes [170]. However, it was recently demonstrated that PGE2 inhibited adipogenesis of 3T3-L1 cells [171, 172]. Epoxyeicosatrienoic acids (EETs), AA metabolites, and AA-derived cytochrome P450 (CYP) epoxygenase metabolites exert anti-inflammatory effects in the vasculature. The expression of CYP2J, a member of P450 subfamily with a role in the bioactivation of AA in extrahepatic tissues, inhibits NF-κB and MAPK signaling pathways and activates of PPARγ, thus reducing IR and diabetic phenotype [173]. n-3 PUFAs, on the other hand, reduce adipose growth and play a role in adipogenesis in various rodent studies [174–183].

Medium-chain fatty acids (MCFAs) (C8–C10) bind the PPARγ ligand binding domain in vitro, causing full inhibition of phosphorylation of PPARγ by cyclin- dependent kinase 5 (cdk5) and reversal of IR in adipose tissue. MCFAs that bind PPARγ also inhibit thiazolidinedione-dependent adipogenesis in vitro [184]. On the other hand, MUFAs were shown to induce adipogenesis and enhance TAG accumulation in 3T3-L1 mouse preadipocytes. Levels of TAGs were greater in cells treated with c-22:1 than c18:1 and c-20:1. Among the c-22:1 fatty acids, c9–22:1 treatment showed higher fat accumulation, associated with increased expression of adipo- genic and lipogenic transcription factors, such as PPARγ and C/EBPα and SREBP-1. However, c-20:1 FAs exhibited less effect than c-18:1 and c-22:1 [185]. Alpha-lipoic acid (ALA) activates insulin signaling pathway and exerts insulin-like properties in adipose and muscle cells. However, 3T3-L1 preadipocytes treated with LA exhibit lower insulin-induced differentiation by modulating activity

and/or expression of various anti-adipogenic transcription factors mainly through activating the MAPK pathways that negatively regulate PPARγ and C/EBPα [141]. 10-oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite, triggered adipocyte differentiation through PPARγ activation and elevated adiponectin secretion and insulin-triggered glucose uptake [142]. Dietary n-3 fatty acids showed more effective activation of PPARα in the liver of rodents [143–145] than n-6 fatty acids [146]. **Figure 3** summarizes the effect of various fatty acid species on the proadipogenic capacity of 3T3L-1 cells in the presence or absence of insulin [147].

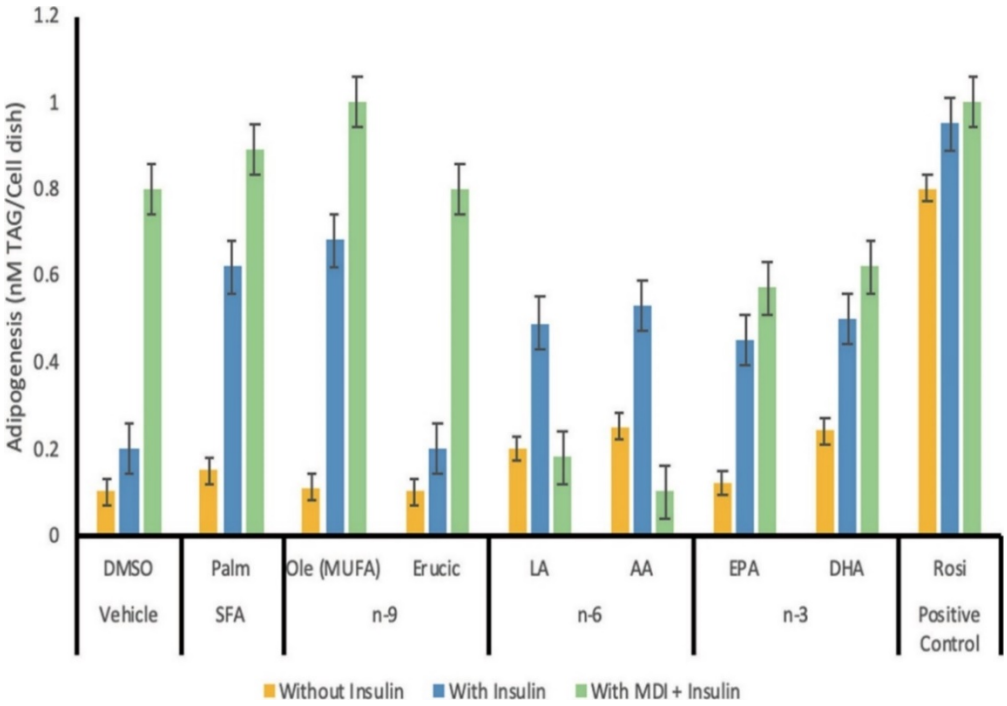

Figure 3. Adipogenic capacity of various fatty acids in 3T3L-1 cells in the absence or presence of 1 µg/ml insulin in differentiation medium (MDI) containing 0.5 mM isobutyl-1-methylxanthine and 1 µM dexamethasone in DMEM and 10% FBS. 100 µM palmitic acid (palm), oleic acid (ole), erucic acid, linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or 1 µM rosiglitazone (rosi) dissolved in DMSO were added when differentiation was induced at day 0 and were present throughout the differentiation period [147].

Lipidomics studies were performed to investigate differences between SAT and VAT depots. These studies have shown evidence of depot-specific enrichment of certain species of TAGs, glycerophospholipids, and sphingolipids and specific correlations between certain lipid species and body mass index, inflammation, and IS [148, 149]. We have recently shown in human SAT and omental (OM) adipose tissue biopsies from 64 obese individuals a number of TAGs that changed with

increased risk IR and T2DM including C46:4, C48:5, C48:4, C38:1, C50:3, C40:2, C56:3, C56:4, C56:7, and C58:7. Enrichment analysis showed C12:0 fatty acid to be associated with TAGs that are least abundant in T2DM. Our data also indicated that C18:3 was present in both depleted and enriched TAGs in T2DM [55]. Secretion of interleukin IL-6 was found to be significantly lower after treatment with C18:2, C22:6, and C16:0 through blocking NF-κB and activating PPARγ [186]. Our data also showed positive correlations between C56:4 and C57:4, both containing C18:2 and C16:0, with SC adipogenic capacity. OM adipogenic capacity was associated with C49:1, C38:0, and C56:2, containing C16:0, C18:1, and C14:0 [55]. **Table 1.** Summarizes a List of TAGs associated with IR, SC and OM adipogenic capacity.TAGs associated with SAT and OM adipogenic capacity. These fatty acids were reported to stimulate adipogenesis in rodents [187–191] and potentially in human preadipocytes.

Table 1. List of TAGs associated with IR, SC, and OM adipogenic capacity

Metabolic Trait	R^2	Importance	TAG	MW	Fatty Acid Composition	Fatty Acids Identities
SC adipogenic	0.9	0.16	C58:10	926.8	C18:2,C18:2,C22:6	linoleic acid,linoleic acid, docosahexaenoic acid
		0.16	C56:4	910.8	C18:1,C18:2,C20:1	oleic acid,linoleic acid, gadoleic acid
		0.14	C57:4	924.7	C22:0,C19:4,C16:0	behenic acid,C19:4,palmitic acid
		0.09	C40:1	692.7	C18:1,C16:0,C6:0	Oleic acid,palmitic acid,caproic acid
		0.08	C60:1	970.8	C24:0,C24:0,C18:1	lignoceric acid,lignocerric acid,oleic acid
		0.22	C38:1	664.7	C18:1,C16:0,C4:0	oleic acid,palmitic acid,butyric acid
OM adipogenic	1	0.18	C48:1	804.8	C18:0,C16:1,C14:0	stearic acid,palmitoleic acid, myristic acid
		0.14	C49:1	818.7	C18:1,C17:0,C14:0	oleic acid,heptadecanoic acid,myristic acid
		0.11	C56:1	916.8	C18:0,C18:0,C20:1	stearic acid,stearic acid,gadoleic
		0.09	C54:0	890.8	C18:0,C18:0,C18:0	stearic acid,stearic acid, stearic acid,
		0.06	C38:0	666.7	C10:0,C14:0,C14:0	capric acid,myristic acid, myristic acid
		0.05	C56:2	914.8	C18:1,C18:1,C20:0	oleic acid,oleic acid,arachidic acid
		0.04	C51:1	846.7	C18:1,C15:0 , C18:0	oleic acid,pentadecanoic acid, stearic acid

4. Environmental factors

Various types of environmental factors were shown to influence adipogenesis. These include environmental pollutants. Among the environmental pollutants, polybrominated diphenyl ethers (PBDEs) represent a widely used type of flame retardants in commercial products and a main source of environmental contaminants. PBDEs accumulate in adipose tissue, potentially changing its endocrine function causing elevation in the risk of IR. We have previously shown that specific congeners of PBDEs (28, 47, 99, and 153) were predominant in VAT from obese individuals and that PBDEs 99, 28, and 47 were elevated in obese IR compared to obese IS. Treatment of human VAT-derived preadipocytes from obese IS individuals with PBDE28 inhibited insulin signaling and reduced adipogenesis [54]. In addition to PBDEs, evidence linking accumulation of other persistent organic pollutants (POPs) and risk of IR and T2DM was previously described [54, 192]. Additionally, the association between inorganic arsenic exposure and the risk of T2DM and obesity was previously reported [193]. Arsenic-induced T2DM is suggested to be mediated by inflammation, oxidative stress, and apoptosis, playing a significant role in the pathogenesis of obesity. Arsenic inhibits adipogenesis and enhances lipolysis, leading to obesity. Other reports have suggested that arsenic may induce lipodystrophy [193]. Another evidence suggests that uremic toxin-treated 3T3-L1 cells and MSC-derived adipocytes exhibit impaired adipogenesis and apoptosis through activation of the Na/K-ATPase/ROS amplification cycle [194]. Other types of environmental pollutants include organotins, widely used antifouling biocides for ships and fishing nets, play a role as endocrine

disruptors as they bind to PPARγ/ RXRα, induce adipogenesis, and repress inflammatory genes in different mammalian cells [195].

5. Conclusion

The pathology of obesity-associated IR and T2DM involves ectopic fat deposition in response to elevated energy intake and poor fat storage. The latter is due to impaired adipogenesis as newly recruited preadicytes become unable to differentiate into fully functional adipocytes. This review presents several factors that influence adipogenesis in pathological obesity including inflammatory media- tors, oxidative stress, fatty acid signaling, and other environmental factors. Most pro-inflammatory cytokines such as IL-6, IL-1β, TNF-α, IL-8, and IFNγ as well as some anti-inflammatory mediators including β-aminoisobutyric acid, A20 enzyme, and EPO have been shown to impair adipogenesis, leading to adipocyte hypertrophy, ectopic fat accumulation, and increased risk of IR and T2DM. However, basal level of adipose tissue inflammation has been shown to be required for normal adipogenesis and functional adipose tissue homeostasis. Similarly, various media- tors of oxidative stress were shown to impact adipogenesis positively such as lipid peroxidation product 4-HNE and negatively such as the marker of oxidative damage 8-OHdG. Targeting lipid peroxidation products was shown to reverse impairment of adipogenesis and sustain IS. However, complete depletion of oxidative stress could also lead to impairment of adipogenesis as basal oxidative stress was shown to be required for normal adipogenesis. Fatty acid signaling also plays a very important role in adipogenesis as various fatty acid species such as PUFAs, MUFAs,

and MCFAs were shown to regulate preadipocyte differentiation at various degrees depending on their composition. Finally, various environmental factors were suggested to impact adipogenesis, mainly through triggering inflammation and oxidative stress, leading to impairment of adipogenesis and increased risk of IR.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in reviewing the literature and preparing and approving the manuscript. MAE is responsible for the integrity of the work as a whole.

Abbreviations

COX-2 cyclooxygenase-2
15-d-PGJ2 15-deoxy-Δ12,14-prostaglandinJ2
4-HNE 4-hydroxynonenal
8-OHdG 8-hydroxy-2-deoxyguanosine
AA arachidonic acid
ATGL adipose triglyceride lipase
BMP4 bone morphogenetic protein 4
C/EBP CCAAT/enhancer-binding protein
CAD Coronary artery disease
cdk5 cyclin-dependent kinase 5
DAGs diacylglycerols
DBC1 deleted in bladder cancer protein 1
DHA docosahexaenoic acid
DMEM dexamethasone

DMSO dimethyl sulfoxide
EETs epoxyeicosatrienoic acids
EPA eicosapentaenoic acid
EPO nonerythropoietic derived peptide
ER endoplasmic reticulum
FABP4 fatty acid-binding protein 4
GIP glucose-dependent insulinotropic polypeptide
HSL hormone-sensitive lipase
IFN-γ interferon-γ
IKKβ inhibitor of nuclear factor kappa-B kinase subunit β IL-6 interleukin 6
IR insulin resistance IS insulin sensitive LA linoleic acid
LPL lipoprotein lipase
LXR liver X receptors
MCFAs medium chain fatty acids
MCP-1 monocyte chemoattractant protein-1 MCPIP Mcp-1-induced protein
MDI insulin in differentiation medium MGL monoglyceride lipase
MHO metabolically healthy obese miRNAs microRNAs MUFAs monounsaturated fatty acids
NF-kappa-B nuclear factor kappa-light-chain enhancer of activated B cells Nnat neurontin
NPGPx nonselenocysteine-containing phospholipid hydroperoxide glutathione peroxidase Ole oleic acid
OM omental adipose tissue Palm palmitic acid
PBDEs diphenyl ethers
PDX protectin DX PGE2 prostaglandin E2
PGF2α prostaglandin F2α
PO pathological obesity
POPs organic pollutants
PPAR peroxisome proliferator-activated receptors PUFAs polyunsaturated

fatty acids ROS reactive oxygen species
Rosi rosiglitazone
SAT subcutaneous adipose tissue
Sirt1 sirtuin 1
Sp1 transcription factor specificity protein 1 SREBP-1c sterol regulatory element binding protein 1C T2DM type 2 diabetes
TAGs triacylglycerolsTNF-α tumor necrosis factor-α TonEBP tonicity-responsive enhancer-binding protein UPR unfolded protein response
VAT visceral adipose tissue
WISP2 inducible-signaling pathway protein 2
ZNF423 zinc finger protein 423
β3-AR beta-3 adrenergic receptor
MSCs mesenchymal stem cells
Ap2 adipocyte protein 2
CYP cytochrome P450
ALA alpha-lipoic acid

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Chapter 3: Metabolic Signature of Obesity-Associated Insulin Resistance and Type 2 Diabetes

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Abstract

Background: Obesity is associated with an increased risk of insulin resistance and type 2 diabetes mellitus (T2DM). However, some obese individuals maintain their insulin sensitivity and exhibit a lower risk of associated comorbidities. The underlying metabolic pathways differentiating obese insulin sensitive (OIS) and obese insulin resistant (OIR) individuals remain unclear.

Methods: In this study, 107 subjects underwent untargeted metabolomics of serum samples using the Metabolon platform. Thirty-two subjects were lean controls whilst 75 subjects were obese including 20 OIS, 41 OIR, and 14 T2DM individuals.

Results: Our results showed that phospholipid metabolites including choline, glycerophosphoethanolamine and glycerophosphorylcholine were significantly altered from OIS when compared with OIR and T2DM individuals. Furthermore, our data confirmed changes in metabolic markers of liver disease, vascular disease and T2DM, such as 3-hydroxymyristate, dimethylarginine and 1,5-anhydroglucitol, respectively.

Conclusion: This pilot data has identified phospholipid metabolites as potential novel biomarkers of obesity-associated insulin sensitivity and confirmed the association of known metabolites with increased risk of obesity-associated insulin resistance, with possible diagnostic and therapeutic applications. Further studies are warranted to confirm these associations in prospective cohorts and to investigate their functionality.

Keywords: Metabolomics, Blood metabolites, Insulin sensitivity, Insulin resistance, Type 2 diabetes Mellitus

Background

Obesity has become a global health care problem due to associated comorbidities including type 2 diabetes mellitus (T2DM), coronary artery disease (CAD), non-alcoholic fatty liver disease (NAFLD) and cancer [1-4]. However, a subset of obese individuals exhibit fewer comorbidities than their equally obese counterparts including maintaining their insulin sensitivity as well as having a healthier lipid profile [5]. The underlying protective mechanisms of the metabolically healthy obesity, also known as insulin sensitive obesity, remain unknown.

Previous studies have suggested that lower levels of inflammatory mediators play a role in the protective phenotype of obese insulin sensitive (OIS) individuals compared to their pathologically obese counterparts, also known as obese insulin resistant (OIR) individuals [6-8]. Other reports have suggested that OIS individuals show fewer markers of oxidative stress [8, 9]. These two mediators (inflammation and oxidative stress) could potentially be influenced by various genetic and environmental factors [10]. Although evidence of the genetic component remains limited, the environmental effect of certain pollutants and various medications has been previously established [11, 12].

Advancement in metabolomic tools including mass spectrometry (MS) technologies has allowed the identification of novel metabolic mediators of disease progression, including obesity associated insulin resistance and T2DM [13]. Recent evidence showed that adipose tissue from OIS, OIR and T2DM individuals exhibit a unique lipidomic signature associated with an increased risk of obesity-associated insulin resistance [14, 15]. Furthermore, metabolomics studies in individuals with T2DM have revealed several diabetes-associated metabolites,

including 1,5-anhydroglucitol (1,5-AG), mannose and glucose [16, 17]. Additionally, lipidomics analysis of plasma samples from young adults has revealed that waist circumference was associated with levels of several sphingomyelins, diacylphosphatidylcholines and lysophosphatidylcholines, whereas HOMA-IR was associated with specific diacylphosphatidylcholines, lysophosphatidylcholines and diacylphosphatidylcholines [18]. However, no metabolomics studies have compared the metabolic differences in blood between lean healthy controls, OIS, OIR and T2DM. Such an approach can provide a deeper understanding of the underlying protective mechanisms in those lower risk individuals, and help in the design of novel diagnostic and therapeutic strategies targeting those at higher risk of disease [19, 20].

The aim of this study was to employ untargeted metabolomics analysis of blood samples from lean, OIS, OIR and obese-T2DM individuals in order to investigate the metabolic pathways underlying obesity-associated insulin resistance and T2DM.

Methods

Materials: Interleukin 6 (IL-6) and leptin ELISAs were from R&D systems (Abingdon, UK). Insulin ELISA was from Mercodia Diagnostics (Uppsala, Sweden). Other chemicals and reagents were from Sigma (Munich, Germany).

Study design: One hundred and seven individuals (75 obese and 32 lean) were recruited at Al Emadi hospital and Hamad Medical Corporation. Lean participants were healthy females visiting the clinic for

acne concerns. Obese participants were amongst patients undergoing weight reduction surgery. Subject inclusion criteria included males and females aged over 18 years and under 65 years of age. Subject exclusion criteria included malignancy or other terminal illness, poorly compliant patients, from whatever cause, inability to give informed consent, or involvement in other research projects. All individuals gave their written informed consent. Protocols were approved by Institutional Review Boards of the Anti-Doping Laboratory Qatar (X2017000224) and Weill Cornell Medicine-Qatar (15-00007). Measurements of body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) were recorded. Fasting blood samples were obtained from all participants. Plasma cholesterol (total, HDL, LDL and triacylglycerol), fasting blood glucose (FBG) and liver function enzymes (total protein, ALP, AST, ALT and bilirubin) were measured by COBAS INTEGRA (Roche Diagnostics, Basil). IL-6, leptin and insulin were determined using commercially available ELISA. Insulin resistance was computed by homeostatic model assessment (HOMA-IR, https://www.dtu.ox.ac.uk/homacalculator/) [21] using 30th percentile (HOMA-IR= 2.4) as a threshold point. Accordingly, obese subjects (BMI > 30) were dichotomized into IS (HOMA-IR < 2.4, n=20, 6 Males and 14 Females), IR (HOMA-IR > 2.4, n=41, 15 Males and 26 Females) and 14 clinically diagnosed T2DM patients (9 Males and 5 Females) according to the definition of the American Diabetes Association (ADA) "Standards of Medical Care in Diabetes" [22].

Metabolomics: Metabolomics profiling was performed using established protocols at Metabolon, Durham, NC, USA. All methods

employed a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The detailed description of the liquid chromatography-mass spectrometry (LC-MS) methodology was previously described [23, 24]. Briefly, serum samples from the 107 participants were methanol extracted to remove the protein fraction. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by hydrophilic interaction chromatography (HILIC)/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Raw data was extracted, peak-identified, and quality control-processed using Metabolon's hardware and software [25]. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities with more than 3300 commercially available purified standard compounds. Library matches for each compound were checked for each sample and corrected if necessary [26].

Statistical analysis of metabolomics data: Statistical analyses were carried out using IBM SPSS version 25, R version 3.2.1 and SIMCA 13.0.1 software (Umetrics, Sweden). Variables with skewed distributions were log transformed or taken the square root of as appropriate to ensure normality [27]. Comparisons were performed with t-test, Wilcoxon–Mann–Whitney and 1-way ANOVA as appropriate. Significance

was defined as $P \leq 0.05$. Non-parametric tests were used for comparing ordinal or non-normal variables. Metabolomics data were log-transformed to ensure normality. Batch correction was performed by Metabolon by rescaling each metabolite's median to 1. Principle component analysis (PCA) was performed using version 2.14, www.r-project.org/. PCA revealed two main components (PC1 and PC2) that together captured 27% of the variance in the data. Linear regression was performed to identify significant metabolites differentiating study groups (OIS vs OIR and T2DM) and (lean=0, OIS=1, OIR=2, T2DM=3, denoting disease progression) using the R statistical package (version 2.14, www.r-project.org/) after correcting for age, gender, BMI and principle components (PC1 and PC2). PCs represent common signals by the metabolites that contribute to the overall variance in the data and uncover fingerprints of confounders allowing their incorporation into the model by assigning them quantifiable measures. In the first model, the variable *study group* is categorical whereas the variable *disease progression* in the second group is continuous. Pathway enrichment analyses were carried out using Chi square tests to identify pathways with metabolites enriched at the top of the list of metabolites ranked by p-value from the linear model since Bonferroni level of significance was not observed. Orthogonal partial least square discriminant analysis (OPLS-DA) was used to compare lean, OIS, OIR and T2DM groups using SIMCA 14 with percentage of missing metabolite values across the samples of 50%. A partial correlation analysis was used to determine metabolic traits of disease (age, BMI, blood pressure, lipids, glucose/insulin/HOMA-IR and liver function enzymes) that exhibit best association with

metabolites showing significantly differing levels between disease groups using IBM SPSS version 25, R version 3.2.1.

Results

General characteristics of participants: Thirty-two lean (BMI=22.7±2.5 kg/m^2, all females) and seventy-five obese and morbidly obese (BMI=45±6.7 kg/m^2, 45 females and 30 males) individuals were recruited at Hamad Medical Corporation and Al Emadi hospital, respectively. Lean individuals were younger and had significantly lower levels of SBP, MAP, triglycerides, triglycerides/HDL ratio, FBG, ALP, ALT and AST than

Table 1. General characteristics of participants

Variables	Lean (N=32) (all F)	OIS (N=20) (4M+16F)	OIR (N=41) (15M+26F)	T2DM (N=14) (9M+5F)	P value ANOVA	All IR (OIR+T2DM) (N=55)	P value OIS vs (OIR + T2DM)
Age (years)	28 (6.8)	35.4 (10.0)	33.17 (10.1)	43 (10.9)	<0.001	35.7 (1.49)	0.92
BMI (kg/m2)	22.7 (2.5)	45.7 (6.038)	45.2 (6.8)	43.3 (7.2)	<0.001	44.8 (0.93)	0.55
SBP (mmHg)	115.3 (13.7)	124.9 (15)	126.9 (19.2)	132.3 (8.3)	0.004	128 (2.32)	0.42
DBP (mmHg)	70.7 (7.8)	74.2 (23.257)	74.0 (11.8)	77.1 (8.8)	0.52	74.8 (1.51)	0.88
MAP (mmHg)	85.6 (8.7)	85.2 (12.82)	91.7 (12.8)	95.8 (8.21)	0.01	92.7 (1.61)	0.03
Cholesterol (mmol/l)	4.3 (0.97)	4.5 (1.24)	4.8 (1.2)	4.9 (0.70)	0.27	4.8 (0.14)	0.24
LDL-cholesterol (mmol/l)	2.5 (0.96)	2.9 (0.89)	3.0 (1.05)	2.8 (0.66)	0.32	3.0 (0.13)	0.74
HDL-cholesterol (mmol/l)	1.4 (0.35)	1.2 (0.36)	1.4 (0.59)	1.2 (0.2)	0.18	1.4 (0.07)	0.12
Triacylglycerol (mmol/l)	0.8 (0.28)	1.1 (0.39)	1.3 (0.62)	1.8 (0.8)	<0.001	1.4 (0.09)	0.04
Triglyceride/HDL	0.7 (0.56)	1.0 (0.45)	1.1 (0.77)	1.6 (1.1)	0.01	1.2 (0.12)	0.28
Leptin (ng/ml)	NA	60.2 (29.9)	51.2 (21.8)	38.9 (23.8)	0.05*	48.0 (3.09)	0.06
Adiponectin (µg/ml)	NA	4.2 (3.19)	3.1 (1.41)	3.4 (1.7)	0.5*	3.1 (0.30)	0.25
IL6 (pg/ml)	NA	3.7 (2.07)	4.3 (2.1)	4.0 (2.0)	0.45*	4.2 (0.27)	0.26
Insulin (pmol/l)	NA	5.3 (1.04)	6.3 (2.7)	11.3 (5.6)	<0.001*	7.6 (0.57)	0.02
FBG (mmol/l)	5.0 (0.39)	6.3 (2.30)	17.9 (8.8)	15.1 (8.6)	<0.001	17.2 (1.18)	<0.001
HOMA-IR	NA	1.5 (0.55)	5.22 (3.2)	6.4 (3.0)	<0.001*	5.5 (0.43)	<0.001
TP (g/l)	73.7 (3.40)	70.3 (4.36)	71 (4.4)	74.3 (7.2)	0.04	71.8 (0.89)	0.34
ALP (U/l)	60.3 (17.1)	70.2 (18.38)	72.5 (16.1)	95.5 (38.1)	<0.001	77.9 (3.46)	0.21
ALT (U/l)	12.6 (5.5)	22.9 (15.16)	31.3 (25.6)	30.9 (19.0)	0.002	31.2 (3.33)	0.15
AST (U/l)	15.5 (4.7)	20.8 (7.84)	24.9 (16.7)	21.7 (10.7)	0.04	24.1 (2.17)	0.36
Bilirubin (µmol/l)	21.2 (4.6)	8.30 (3.84)	8.2 (4.4)	8.5 (3.5)	0.55	8.0 (0.60)	0.9

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, MAP mean arterial blood pressure, LDL low density lipoprotein, HDL high density lipoprotein, IL-6 interleukin 6, FBG fasting blood glucose, HOMA-IR homeostatic model assessment of insulin resistance, TP total protein, ALP alkaline phosphatase, ALT alanine transaminase, AST aspartate aminotransferase, F female, M male. Data are presented as mean (SD). Differences between OIS, OIR and T2DM were tested by ANOVA. Differences between (OIS and OIR) and (OIS vs OIR+T2DM) were tested by independent sample t test (normally distributed variables) or Mann–Whitney U (variables with skewed distribution) test. A p-value significance level of 0.05 was used. The asterisk (*) denotes ANOVA that compared OIS, OIR and T2DM due to lack of data from the lean group

obese individuals. Among obese participants, OIR individuals showed higher FBG than expected, suggesting a high prevalence of undiagnosed T2DM within this group. Therefore, subsequent analyses considered OIR and T2DM groups as one group (all IR) as both groups share obesity and insulin resistance. OIS subjects showed significantly lower MAP and levels of triacylglycerols, FBG, insulin and HOMA-IR than their equally obese all IR (OIR+T2DM) counterparts (Table 1).

Metabolites differentiating OIS from OIR +T2DM: Non-targeted metabolomics of serum samples from the 107 participants was applied to identify metabolites that differentiate OIS vs OIR and OIS vs OIR+T2DM individuals to reveal a metabolic signature of obesity-associated insulin resistance and T2DM. Initial analysis revealed no significant differences in levels of metabolites between OIS and OIR due to their small group sizes (data not shown); however, when combining OIR+T2DM, the linear model revealed 27 metabolites exhibiting significant differences between OIS and OIR+T2DM groups (Table 2). These included metabolites associated with glycolysis, gluconeogenesis and pyruvate metabolism (glucose and 1,5 AG), histidine metabolism (1-methylhistamine, 1-ribosyl-imidazoleacetate and formiminoglutamate) and phospholipid metabolism (choline, glycerophosphoethanolamine and glycerophosphorylcholine). Since the Bonferroni level of significance was not achieved for any of the identified associations, pathway enrichment analysis was performed based on identifying pathways reported by nominally significant metabolites more frequently than can be attributed to random chance. Among the significantly altered

metabolic pathways, the phospholipids metabolic pathway was significantly over-represented based on enrichment analysis of the nominally significant metabolites from the group comparisons (p=3.9E-7). The corresponding metabolites associated with the phospholipids metabolic pathway differentiating OIS from OIR+T2DM included choline, glycerophosphoethanolamine and glycerophosphorylcholine (GPC) (highlighted in Table 2). Figure 1 illustrates levels of significant metabolites that belong to enriched pathways in different study groups. Figure 1 demonstrates higher levels of choline, glycophosphoethanolamine and GPC in OIS compared OIR+T2DM. Levels of these metabolites in individual groups are also shown in Additional **file1: Fig . S1 .**

Table 2. Metabolites differentiating OIS from OIR+T2DM. Highlighted rows reprsent metabolites that belong to the significantly enriched phospholipids pathway.

Metabolites	Sub pathway	Super pathway	Fold change	Std. error	p value
1,5-Anhydroglucitol (1,5-Ag)	Glycolysis gluconeogenesis and pyruvate metabolism	Carbohydrate	− 0.92	0.4	0.041
12-Dilinoleoyl-Gpc (18:2/18:2)	Phosphatidylcholine (PC)	Lipid	0.47	0.2	0.037
12-Dilinoleoyl-Gpe (18:2/18:2)*	Phosphatidylethanolamine (PE)	Lipid	1.23	0.51	0.037
1-Methylhistamine	Histidine metabolism	Amino Acid	1.16	0.38	0.007
1-Ribosyl-Imidazoleacetate*	Histidine metabolism	Amino Acid	0.85	0.35	0.03
26-Dihydroxybenzoic Acid	Drug—topical agents	Xenobiotics	1.18	0.42	0.011
3-Amino-2-Piperidone	Urea cycle; arginine and Proline metabolism	Amino Acid	− 0.99	0.37	0.016
5-Methylthioadenosine	Polyamine metabolism	Amino Acid	1.18	0.38	0.006
Alpha-hydroxyisovalerate	Leucine isoleucine and valine metabolism	Amino Acid	0.7	0.27	0.023
Arachidonoylcholine	Fatty acid metabolism (acyl choline)	Lipid	− 1.05	0.41	0.021
Choline	*Phospholipid metabolism*	*Lipid*	*− 0.46*	*0.17*	*0.013*
Cortisol	Corticosteroids	Lipid	0.87	0.31	0.012
Docosatrienoate (22:3N3)	Long chain polyunsaturated fatty acid (n3 and n6)	Lipid	0.64	0.25	0.024
Formiminoglutamate	Histidine metabolism	Amino Acid	1.15	0.52	0.05
Gamma-tocopherol/beta-tocopherol	Tocopherol metabolism	Cofactors and Vitamins	1.38	0.55	0.024
Glucose	Glycolysis gluconeogenesis and pyruvate metabolism	Carbohydrate	0.3	0.12	0.025
Glycerol 3-phosphate	Glycerolipid metabolism	Lipid	− 0.93	0.38	0.029
Glycerophosphoethanolamine	Phospholipid metabolism	Lipid	− 1.24	0.47	0.019
Glycerophosphorylcholine (GPC)	Phospholipid metabolism	Lipid	− 1.85	0.77	0.032
HWESASXX*	Tyrosine metabolism	Amino Acid	− 1.44	0.59	0.029
Methionine sulfone	Drug—metabolic	Xenobiotics	0.89	0.34	0.018
Methylphosphate	Benzoate Metabolism	Xenobiotics	− 1.55	0.48	0.006
N-Formylanthranilic Acid	Fatty acid metabolism (acyl carnitine monounsaturated)	Lipid	0.9	0.4	0.044
N-Stearoyl-Sphinganine (D18:0/18:0)*	Endocannabinoid	Lipid	0.84	0.31	0.015
N-Stearoyl-Sphingosine (D18:1/18:0)*	Ceramides	Lipid	0.44	0.2	0.05
Pipecolate	Fatty acid dicarboxylate	Lipid	0.92	0.32	0.011
Ribitol	Vitamin A metabolism	Cofactors and vitamins	0.33	0.14	0.044

Italicized rows represent metabolites that belong to the significantly enriched phospholipids pathway. Linear regression was performed to identify significant metabolites differentiating OIS from OIR and T2DM using the R statistical package after correcting for age, gender, BMI and principle components (PC1 and PC2). A p-value significance level of 0.05 was used. Asterisks (*) on IDs of some metabolites indicate that they have not been officially confirmed based on a standard, but their identities are known with confidence[23].

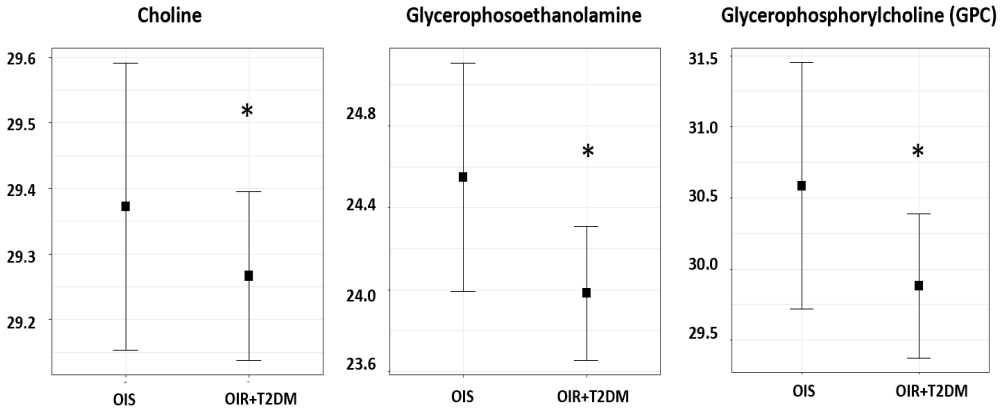

Figure 1. Boxplot of metabolites that belong to the enriched phospholipid pathway differentiating OIS and OIR+T2DM groups. Linear regression was performed to identify significant metabolites differentiating OIS from OIR and T2DM using the R statistical package after correcting for age, gender, BMI and principle components (PC1 and PC2). Y-axis indicates levels of metabolites (log2). * p-value significance level of 0.05 was used.

Metabolites associated with disease progression: An additional a linear model was used to assess the significance of metabolites associated with increased risk of obesity-associated insulin resistance and T2DM (as defined in the method section). Sixty-six metabolites exhibited significant differences with disease progression. The list of metabolites and their associated pathways are shown in Additional file 2 : Table S1. These included metabolites associated with glycolysis (glucose), mannose metabolism (mannose), monohydroxy fatty acid (3-hydroxylaurate, 3-hydroxyoctanoate, 3-hydroxydecanoate and 3-hydroxymyristate), medium chain fatty acids (laurate) and urea cycle; arginine and proline metabolism (ADMA + SDMA) among others. Enriched metabolic pathways included glycolysis, gluconeogenesis and the pyruvate metabolic pathway (p=0.02), fatty acid monohydroxy metabolic pathway, urea cycle metabolic pathway (p=0.04) and

arginine and proline metabolic pathway (p=0.05). Subsequently, metabolites that showed significant differences with disease progression (Additional file 2: Table S1) within these enriched pathways were identified (Table 3). Figure 2 demonstrates patterns of increased(3-hydroxylaurate,3hydrocyoctanoate,3hydroxydecanoate,3-hdroxymyristate, and glucose) or decreased (1,5-AG, ADMA+SDMA, homoarginine, ornithine, 2-oxoarginine) metabolites with disease progression.

Table 3. Metabolites that belong to the significantly enriched pathways associated with obesity-associated insulin resistance and T2DM.

Metabolites	Sub pathway	Super pathway	Beta value	Std. error	p value
3-Hydroxylaurate	Fatty acid monohydroxy	Lipid	0.3	0.1	0.002
3-Hydroxyoctanoate		Lipid	0.3	0.1	0.008
3-Hydroxydecanoate		Lipid	0.3	0.1	0.009
3-Hydroxymyristate		Lipid	0.2	0.1	0.012
Glucose	Glycolysis gluconeogenesis and pyruvate metabolism	Carbohydrate	0.2	0	0.001
15-Anhydroglucitol (1,5-AG)		Carbohydrate	− 0.4	0.2	0.019
Dimethylarginine (ADMA + SDMA)	Urea cycle; arginine and proline metabolism	Amino Acid	− 0.3	0.1	0.009
Homoarginine		Amino Acid	− 0.3	0.1	0.017
Ornithine		Amino Acid	− 0.2	0.1	0.042
2-Oxoarginine*		Amino acid	− 0.3	0.1	0.045

Linear regression was performed to identify significant metabolites associated with disease progression (lean, OIS, OIR, T2DM) using the R statistical package after correcting for age, gender, BMI and principle components (PC1 and PC2). A p-value significance level of 0.05 was used. Asterisks (*) on IDs of some metabolites indicate that they have not been officially confirmed based on a standard, but their identities are known with confidence [23].

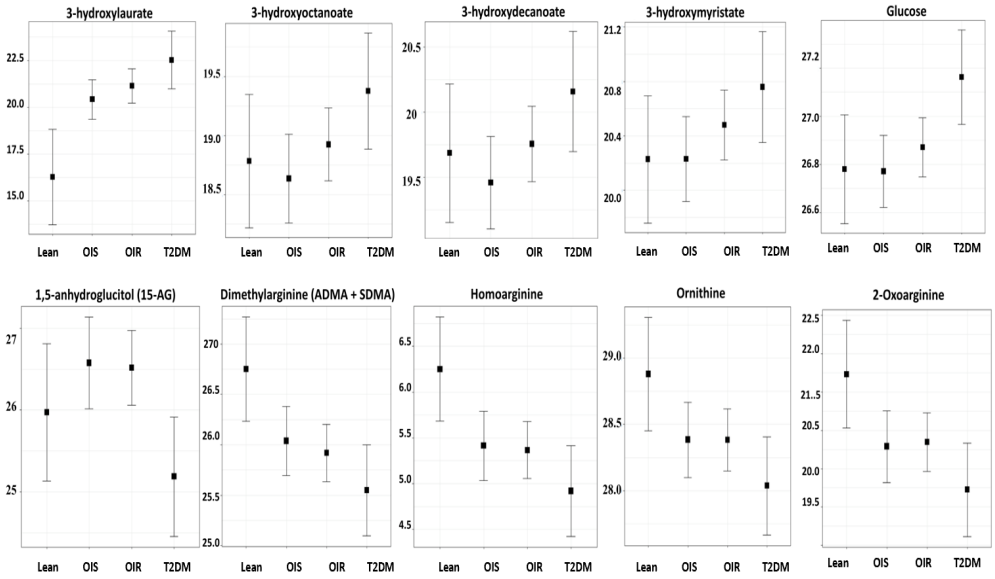

Figure 2. Boxplot of metabolites that belong to the enriched pathways associated with increased risk of obesity-associated insulin resistance and T2DM. Linear regression was performed to identify significant metabolites associated with disease progression using the R statistical package after correcting for age, gender, BMI and principle components (PC1 and PC2). Y-axis indicates levels of metabolites (log2). A p-value significance level of 0.05 was used.

An orthogonal partial least square discriminate analysis (OPLS-DA) comparing subjects from lean, OIS, OIR and T2DM was used for ease of visualization. The model revealed three class-discriminatory components accounting for 48% of the variation in the data due to participant groups (Figure 3). The score plot in Figure 3A indicates an x-axis separating the lean group from OIS, OIR and T2DM; the latter group being rather separated along the y-axis. The corresponding loading plot, shown in Figure 3B, indicates enriched pathways' associated metabolites significantly differentiating OIS and OIR+T2DM and those associated with disease progression as per linear models. Specifically, higher glucose, choline, GPC, 3-hydroxymyristate and 3-hydroxylaurate and

lower 1,5-AG, dimethylarginine (ADMA + SDMA), homoarginine, ornithine and 2-oxoarginine are indicated.

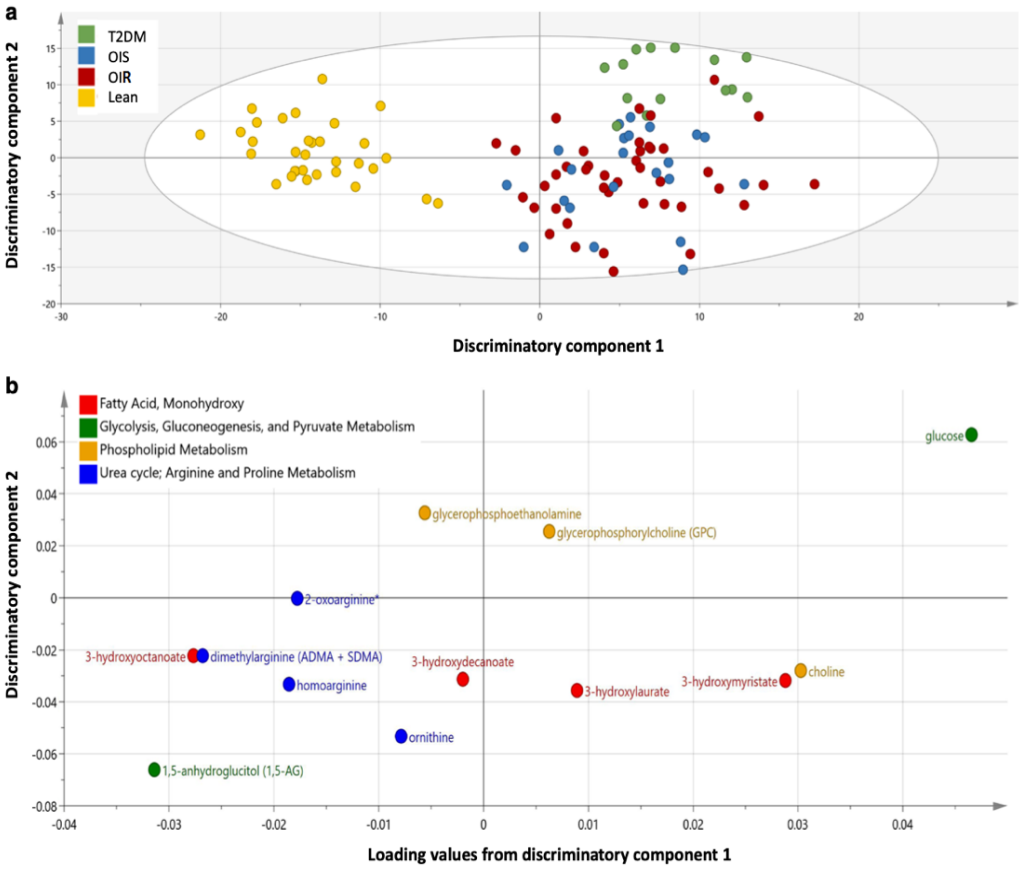

Figure 3. OPLS-DA model comparing metabolites from lean, OIS, OIR and T2DM individuals. (a) A score plot showing the class-discriminatory component 1 (*x*-axis) versus class-discriminatory component 2 (*y*-axis). (b) The corresponding loading plot showing enriched pathways' associated metabolites differentiating OIS and OIR+T2DM groups or those associated with disease progression.

Correlation of significant metabolites with mediators of metabolic disease: A partial correlation analysis was used to determine traits of disease best associated with metabolites showing significantly differing levels between disease groups. In essence, the correlation between each of such metabolites and each trait was evaluated after correcting for the effect of all other remaining traits. The correlations that remained significant after such correction are listed in Table 4. The trait of liver function enzymes (ALP and ALT), BMI, TAGs, leptin, insulin and HOMA-IR showed the most significant correlations with levels of metabolites differentiating OIS and OIR+T2DM and those associated with disease progression.

Table 4. Classical metabolic traits that best predict levels of metabolites differentiating OIS and OIR+T2DM and those associated with disease progression.

Metabolite	Partial correlation (R)	Mediator of metabolic disease	p value
3-Hydroxylaurate	0.4	ALP	0.003
3-Hydroxyoctanoate	0.4	ALP	0.002
		BMI	0.003
3-Hydroxydecanoate	0.83	BMI	< 0.001
Glucose	0.4	Insulin	< 0.001
		TAGs	0.001
1,5-Anhydroglucitol (1,5-AG)	− 0.4	Insulin	< 0.001
		TAGs	< 0.001
		HOMA-IR	0.004
Ornithine	0.4	ALT	< 0.001
		Leptin	< 0.001
Choline	0.4	ALT	< 0.001
		Leptin	0.001
Glycerophosphoethanolamine	0.39	BMI	< 0.001

A partial correlation analysis by stepwise linear regression was performed using IBM SPSS version 25, R version 3.2.1. A p-value significance level of 0.005 was used.

Discussion

Obesity triggers a cascade of biochemical changes that increase the risk of various comorbidities including insulin resistance and T2DM. However, some obese individuals seem to be protected against obesity-associated comorbidities. Understanding the underlying mechanisms of this apparently protective phenotype could provide a therapeutic strategy to mitigate the comorbidities associated with pathological obesity. Various studies have investigated the potential mechanisms underlying differences among lean, obese-IS, obese-IR and obese-T2DM individuals [7, 9, 11, 14, 28, 29], however no study has compared the differences in the metabolic signature among these groups as a means to identify potential diagnostic and therapeutic targets. In this study, untargeted metabolomics analysis of serum samples from lean, OIS, OIR and obese-T2DM individuals was utilized to investigate the metabolic pathways underlying progression of insulin resistance and T2DM. Our novel data indicate that the phospholipid metabolites (choline, glycerophosphoethanolamine and glycerophosphorylcholine) were significantly altered when comparing OIS and OIR + T2DM. Additionally, our data confirmed metabolic changes in several metabolic pathways with obesity-associated insulin resistance and T2DM, including fatty acid and arginine metabolism as well as metabolic markers of liver disease, vascular disease, and diabetes. Therefore, the novel metabolites reported here differentiate the metabolically healthy obese group (OIS) from the pathological obese group (OIR + T2DM) and confirm known biomarkers of obesity-associated insulin resistance with potential diagnostic and therapeutic applications. The causative nature of the

identified correlations between metabolites and insulin resistance cannot be ruled out particularly as it is recognized that free fatty acids, for instance, increase insulin resistance [30]. Therefore, future in vitro and in vivo functional studies are warranted where the effects of these metabolites on inducing insulin resistance could confirm their functional relevance.

A novel metabolic signature differentiating OIS and OIR + T2DM

Since there were no difference between OIS and OIR likely due to their small group sizes, the analysis was repeated by comparing OIS and combined OIR and T2DM groups as the latter two groups were matched for obesity and insulin resistance. Three phospholipids were found to differentiate between OIS and OIR + T2DM. These included increased levels of choline and GPC in OIS compared to OIR + T2DM and lean groups, suggesting a protective role in obesity-associated insulin resistance. GPC is a natural precursor of phospholipids and a metabolite derived from phosphatidylcholine. It contributes the most to circulating choline levels; therefore, GPC serves as a precursor for acetylcholine. The latter is an important neurotransmitter and a vasodilator that shows a different microvascular reactivity between IR and IS nondiabetic women [31]. Previous studies have reported that dietary choline levels can also lower the risk of fatty liver disease and liver damage [32]. Glycerophosphoethanolamine was another metabolite that differentiated OIS from OIR + T2DM. Glycerophosphoethanolamine represents a membrane degradation product that has been linked to chronic liver disease [33]. The novel associations between higher levels of these phospholipid metabolites and obesity-associated insulin sensitivity

could therefore reflect decreased risk of microvascular disease, small vessel disease, lipotoxic cardiac diseases and non-alcoholic liver disease in the OIS group compared to OIR + T2DM group of participants [34,35,36].

Metabolic signature of obesity-associated insulin resistance and T2DM

When comparing the metabolic profiles of lean, obese-IS, IR and T2DM individuals, several metabolites significantly changed with disease progression. These included metabolites that were previously reported in association with insulin resistance and T2DM such as glucose and 1,5-AG [37, 38]. Other identified metabolites were reported in association with comorbidities of insulin resistance and T2DM including fatty acid metabolic disorders (such as 3-hydroxylaurate) [39], impairment of liver function and diabetic status (such as 3-hydroxymyristate and homoarginine) [40, 41] and vascular disease (such as dimethylarginine) [42]. Other novel arginine metabolites were also found to be significantly changed with disease progression including ornithine (a precursor of arginine, also a medication for hepatic encephalopathy) [43] and 2-oxoarginine (a metabolite of arginine catabolism and a marker of argininemia) [44]. Novel metabolites in association with disease progression were also identified including medium chain fatty acids 3-hydroxyoctanoate and 3-hydroxydecanoate that have been reported to be involved in beta-oxidation of longer-chain fatty acids [45, 46]. Previous reports have associated increased plasma levels of 3-hydroxyoctanoate in patients with an inherited deficiency of long-chain 3-hydroxyacyl-CoA dehydrogenase, as a marker of various clinical cases such as

recurrent myoglobinuria, hypoketotic hypoglycemic encephalopathy, hypertrophic/dilatative cardiomyopathy, sudden infant death, and fulminant hepatic failure [46, 47].

Correlation between metabolites differentiating OIS and OIR + T2DM and classical mediators of metabolic disease

When considering correlations between the identified metabolites and classical mediators of metabolic disease such as age, BMI, lipids, FBG, insulin, HOMA-IR and liver function enzymes, a partial correlation analysis revealed several significant associations. Choline, previously shown to be lower in hepatic damage [48], was found to positively correlate with leptin and ALT. Despite its positive correlation with ALT, choline was found to be higher in OIS compared to OIR + T2DM, indicating a relationship between this metabolite and the protective phenotype of OIS individuals that requires further investigation. On the other hand, glycerophosphoethanolamine was found to be associated with BMI, suggesting increased levels of this membrane degradation product with obesity.

Correlation of disease progression metabolites and classical mediators of metabolic disease

As expected, glucose and 1,5-AG, previously shown to be associated with T2DM, were found to correlate significantly with levels of insulin and circulating triacylglycerol levels. When considering metabolites that were significantly associated with obesity-related comorbidities, a significant correlation between levels of 3-hydroxylaurate and ALP, was revealed. This suggests that 3-hydroxylaurate, a medium chain fatty

acid that is associated with intolerance to prolonged fasting and recurrent episodes of hypoglycemic coma, may constitute a novel marker of fatty liver disease. Similarly, 3-hydroxyocanoate was also found to be associated with ALP and BMI, suggesting that it may also be a novel marker of obesity-associated fatty liver disease. 3-hydroxydecanoate was also found to be associated with BMI, suggesting increased levels of another medium-chain fatty acid with a role in the beta-oxidation and obesity. Ornithine, previously shown to be associated with hepatic damage, was found to be associated with leptin and ALT, providing a further evidence of its association with obesity associated non-alcoholic fatty liver disease.

Study limitations. This has a number of limitations including the relatively low number of participants per group and the cross-sectional nature of the study limited the interpretation of the findings from a pathophysiological point of view. The observational nature of the findings requires functional validation before suggesting any causalities, especially as some findings were based on weak to moderate associations. Furthermore, since blood samples were collected at multiple sites, a batch effect may have occurred, but this was mitigated by standardized protocols for sample collection, processing and storage. It is possible that other unmeasured factors may have impacted our data including dietary habits, medication/supplements and other unknown environmental factors; however, inclusion of principle components in the regression model may have captured part of these potential confounding factors. Finally, controls were not matched for age and gender compared to the study groups, adding an additional variable; however, both

age and gender were corrected for in the analysis, but their influence over metabolic differences cannot be ruled out.

Conclusion

In the comparison between equally obese insulin sensitive and insulin resistance individuals, phospholipid metabolites including choline, glycerophosphoethanolamine and glycerophosphorylcholine (GPC) were significantly altered. In addition, several metabolites were identified and were confirmatory for insulin resistance and T2DM (such as glucose and 1,5-AG) or their comorbidities (such as 3-hydroxylaurate, 3-hydroxymyristate, homoarginine and dimethylarginine). This pilot study also identified novel metabolic markers such as the medium chain fatty acids 3-hydroxyoctanoate and 3-hydroxydecanoate and highlighted their potential link to non-alcoholic fatty liver disease, a hallmark of increased risk of obesity-associated insulin resistance. Further studies are needed to confirm these associations in prospective cohorts and to investigate their functional relevance.

List of abbreviations

Body mass index (BMI)
Diastolic blood pressure (DBP)
Fasting blood glucose (FBG),
High density lipoprotein (HDL)
Homeostatic model assessment (HOMA-IR)
Interleukin 6 (IL-6)
LDL (low density lipoprotein),
MAP (mean arterial blood pressure),
Non-aqueous reverse phase UHPLC separation (NARP)
Obese insulin sensitive (OIS)
Obese insulin resistant (OIR)
Omental (OM)
Orthogonal partial least square discriminate analysis (OPLS-DA)
Principle component analysis (PCA)
Systolic blood pressure (SBP)
Triacylglycerols (TAGs)
Type 2 diabetes mellitus (T2DM)
Ultra-high-performance liquid chromatography (UHPLC)
Coronary artery disease (CAD)
mass spectrometry (MS)
1,5-anhydroglucitol (1,5-AG),
ultra-performance liquid chromatography (UPLC)
heated electrospray ionization (HESI-II)
liquid chromatography-mass spectrometry (LC-MS)
hydrophilic interaction chromatography (HILIC)
Asymmetric dimethylarginine (ADMA)
Symmetric dimethylarginine (SDMA)

Declarations

Ethics approval and consent to participate

All participants provided informed consent. Protocols were approved by Institutional Review Boards of Ani Doping Lab Qatar (X2017000224) and Weill Cornell Medicien-Qatar (15-00007)

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

HS carried out most of the sample preparation, data acquisition and analysis, helped with drafting the article and approved the final version. ID carried out the statistical analysis and helped with data interpretation. MA, AD, FM, and SA contributed to the study design, sample collection and data analysis. MAE and NAM were lead principle investigators, designed the experiments, supervised progress, analyzed data and wrote and approved the final version of the article. MAE and NAM are responsible for the integrity of the work as a whole.

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Table S1. Metabolites associated with disease progression

Metabolites	Sub pathway	Super pathway	Estimate	Std. Error	t value	P value
Glucose	Glycolysis Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	0.15	0.04	3.4	0.001
Palmitoleamide (16:1)*	Fatty Acid Amide	Lipid	-1.97	0.00	-506.8	0.001
3-(2-Hydroxyphenyl)Propionate	Benzoate Metabolism	Xenobiotics	-1.06	0.28	-3.7	0.002
Cyclosporin A	Drug - Antiinflammatory Immunosuppressant	Xenobiotics	1.29	0.38	3.3	0.002
3-Hydroxylaurate	Fatty Acid Monohydroxy	Lipid	0.32	0.10	3.1	0.002
Mannose	Fructose Mannose and Galactose Metabolism	Carbohydrate	0.21	0.07	3.1	0.003
Heme	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	-0.93	0.31	-3.1	0.003
N6N6N6-Trimethyllysine	Lysine Metabolism	Amino Acid	-0.44	0.15	-3.0	0.003
2'-Deoxyuridine	Pyrimidine Metabolism Uracil containing	Nucleotide	-0.32	0.11	-2.8	0.006
3-Hydroxyoctanoate	Fatty Acid Monohydroxy	Lipid	0.30	0.11	2.7	0.008
Glycerophosphoserine*	Phospholipid Metabolism	Lipid	-0.57	0.21	-2.7	0.009
Dimethylarginine (Adma + Sdma)	Urea cycle; Arginine and Proline Metabolism	Amino Acid	-0.27	0.10	-2.7	0.009
Isoleucylglycine	Dipeptide	Peptide	-0.65	0.24	-2.7	0.009
3-Hydroxydecanoate	Fatty Acid Monohydroxy	Lipid	0.27	0.10	2.7	0.009
N-Acetylhistidine	Histidine Metabolism	Amino Acid	-0.26	0.10	-2.6	0.010
Phosphate	Oxidative Phosphorylation	Energy	0.25	0.10	2.6	0.011
Laurate (12:0)	Medium Chain Fatty Acid	Lipid	-0.30	0.12	-2.6	0.012
3-Hydroxymyristate	Fatty Acid Monohydroxy	Lipid	0.23	0.09	2.6	0.012
26-Dihydroxybenzoic Acid	Drug - Topical Agents	Xenobiotics	0.41	0.16	2.6	0.012
Tyramine O-Sulfate	Tyrosine Metabolism	Amino Acid	-0.98	0.38	-2.5	0.013
Dodecanedioate (C12)	Fatty Acid Dicarboxylate	Lipid	-0.36	0.14	-2.5	0.015
1-Methylhistidine	Histidine Metabolism	Amino Acid	-0.33	0.13	-2.5	0.015
Lidocaine	Drug - Analgesics Anesthetics	Xenobiotics	-1.86	0.73	-2.5	0.015
Aconitate [Cis Or Trans]	TCA Cycle	Energy	0.14	0.06	2.5	0.015
Homoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	-0.27	0.11	-2.42	0.017
Stearoylcholine*	Fatty Acid Metabolism (Acyl Choline)	Lipid	-0.41	0.17	-2.42	0.018
Kynurenine	Tryptophan Metabolism	Amino Acid	-0.19	0.08	-2.40	0.019
Spermidine	Polyamine Metabolism	Amino Acid	-0.50	0.21	-2.39	0.019

15-Anhydroglucitol (15-Ag)	Glycolysis Gluconeogenesis and Pyruvate Metabolism	Carbohydrate	-0.41	0.17	-2.38	0.019
Tryptophan	Tryptophan Metabolism	Amino Acid	-0.14	0.06	-2.38	0.019
N-Acetylhistamine	Histidine Metabolism	Amino Acid	1.31	0.51	2.59	0.020
Trizma Acetate	Chemical	Xenobiotics	1.96	0.83	2.38	0.024
2-Methoxyacetaminophen Glucuronide*	Drug - Analgesics Anesthetics	Xenobiotics	-0.78	0.33	-2.34	0.024
Myristoyl-Linoleoyl-Glycerol (14:0/18:2) [1]*	Diacylglycerol	Lipid	-0.35	0.15	-2.29	0.025
Docosadioate (C22-Dc)	Fatty Acid Dicarboxylate	Lipid	0.31	0.14	2.28	0.025
N-Stearoyl-Sphingadienine (D18:2/18:0)*	Ceramides	Lipid	0.20	0.09	2.27	0.026
N-Stearoylserine*	Endocannabinoid	Lipid	-0.24	0.11	-2.26	0.026
Thromboxane B2	Eicosanoid	Lipid	-2.23	0.90	-2.47	0.026
Isoursodeoxycholate	Secondary Bile Acid Metabolism	Lipid	0.73	0.33	2.25	0.027
Gamma-Tocopherol/Beta-Tocopherol	Tocopherol Metabolism	Cofactors and Vitamins	0.49	0.22	2.25	0.027
Diacylglycerol (12:0/18:1 14:0/16:1 16:0/14:1) [1]*	Diacylglycerol	Lipid	-0.58	0.26	-2.25	0.027
Sphinganine-1-Phosphate	Sphingolipid Synthesis	Lipid	-0.37	0.16	-2.25	0.027
Picolinate	Tryptophan Metabolism	Amino Acid	-0.30	0.13	-2.24	0.028
Behenoylcarnitine (C22)*	Fatty Acid Metabolism (Acyl Carnitine Long Chain Saturated)	Lipid	-0.31	0.14	-2.21	0.032
Linoleoylcholine*	Fatty Acid Metabolism (Acyl Choline)	Lipid	-0.33	0.15	-2.17	0.032
Cys-Gly Oxidized	Glutathione Metabolism	Amino Acid	-0.43	0.20	-2.16	0.034
Glycochenodeoxycholate Glucuronide (1)	Primary Bile Acid Metabolism	Lipid	-0.57	0.27	-2.13	0.036
Adipate	Fatty Acid Dicarboxylate	Lipid	-0.27	0.13	-2.12	0.037
7-Methylguanine	Purine Metabolism Guanine containing	Nucleotide	-0.11	0.05	-2.11	0.037
N6N6-Dimethyllysine	Lysine Metabolism	Amino Acid	-0.31	0.15	-2.11	0.037
N-Stearoyl-Sphingosine (D18:1/18:0)*	Ceramides	Lipid	0.17	0.08	2.10	0.038
1-Linoleoyl-2-Arachidonoyl-Gpe (18:2/20:4)*	Phosphatidylethanolamine (PE)	Lipid	0.35	0.16	2.12	0.039
Ethylmalonate	Leucine Isoleucine and Valine Metabolism	Amino Acid	0.26	0.13	2.10	0.039
Butyrate (4:0)	Short Chain Fatty Acid	Lipid	-0.41	0.19	-2.10	0.039
Hydroxy-N6N6N6-Trimethyllysine*	Lysine Metabolism	Amino Acid	-0.23	0.11	-2.08	0.040

N-Acetylglutamine	Glutamate Metabolism	Amino Acid	-0.26	0.12	-2.07	0.041
1-Palmitoyl-2-Alpha-Linolenoyl-Gpc (16:0/18:3N3)*	Phosphatidylcholine (PC)	Lipid	0.23	0.11	2.07	0.041
Dihydrocaffeate Sulfate (2)	Food Component/Plant	Xenobiotics	0.68	0.33	2.07	0.042
Ornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	-0.17	0.08	-2.06	0.042
Orotidine	Pyrimidine Metabolism Orotate containing	Nucleotide	-0.23	0.11	-2.04	0.045
2-Oxoarginine*	Urea cycle; Arginine and Proline Metabolism	Amino Acid	-0.28	0.14	-2.03	0.045
S-Adenosylhomocysteine (Sah)	Methionine Cysteine SAM and Taurine Metabolism	Amino Acid	-0.23	0.11	-2.03	0.045
Sulfate*	Chemical	Xenobiotics	0.10	0.05	2.02	0.046
Quinolinate	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	-0.22	0.11	-2.02	0.047
3-Methoxycatechol Sulfate (1)	Benzoate Metabolism	Xenobiotics	0.94	0.46	2.03	0.047
5-Acetylamino-6-Amino-3-Methyluracil	Xanthine Metabolism	Xenobiotics	0.44	0.22	2.00	0.049

Chapter 4: Triglyceride Profiling in Adipose Tissues from Obese Insulin Sensitive, Insulin Resistant and Type 2 Diabetes Mellitus Individuals

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Abstract

Background: Lipid intermediates produced during triacylglycerols (TAGs) synthesis and lipolysis in adipocytes interfere with the intracellular insulin signaling pathway and development of insulin resistance. This study aims to compare TAG species and their fatty acid composition in adipose tissues from insulin sensitive (IS), insulin resistant (IR) and type 2 diabetes mellitus (T2DM) obese individuals.

Methods: Human subcutaneous and omental adipose tissue biopsies were obtained from 64 clinically characterized obese individuals during weight reduction surgery. TAGs were extracted from the adipose tissues using the Bligh & Dyer method, then were subjected to non-aqueous reverse phase ultra-high performance liquid chromatography and full scan mass spectrometry acquisition and data dependent MS/MS on LTQ dual cell linear ion trap. TAGs and their fatty acid contents were identified and compared between IS, IR and T2DM individuals and their levels were correlated with metabolic traits of participants and the adipogenic potential of preadipocyte cultures established from their adipose tissues.

Results: Data revealed 76 unique TAG species in adipose tissues identified based on their exact mass. Analysis of TAG levels revealed a number of TAGs that were significantly altered with disease progression including C46:4, C48:5, C48:4, C38:1, C50:3, C40:2, C56:3, C56:4, C56:7 and C58:7. Enrichment analysis revealed C12:0 fatty acid to be associated with TAGs least abundant in T2DM whereas C18:3 was found in both depleted and enriched TAGs in T2DM. Significant correlations of various adipose tissue-derived TAG species and metabolic traits were observed, including age and body mass index, systemic total cholesterol, TAGs, and interleukin-6 in addition to adipogenic potential of preadipocytes derived from the same adipose tissues.

Conclusion: Pilot data suggest that adipose tissues from obese IR and T2DM individuals exhibit TAG-specific signatures that may contribute to their increased risk compared to their IS counterparts. Future experiments are warranted to investigate the functional relevance of these specific lipidomic profiles.

Keywords: Lipidomics, Adipose tissue, Triaclyglycerols, Insulin sensitivity, Insulin resistance, Type 2 diabetes Mellitus

Background

Adipose tissue is the main site for storing and mobilizing energy in response to metabolic demand. Obesity is associated with changes in the structure and function of the adipose tissue, leading to progression of insulin resistance and type 2 diabetes mellitus (T2DM) [1]. However, a subset of obese individuals, known as the insulin sensitive (IS) obese, maintain insulin sensitivity and exhibit better adipose tissue functions compared to equally obese insulin resistant (IR) counterparts [2]. Obesity triggers hypertrophy of adipocytes within the subcutaneous (SC) adipose tissues to enable accumulation of excess triacylglycerols (TAGs). Additional energy intake causes further fat accumulation within the omental (OM) depot, which is associated with ectopic fat deposition in the liver, skeletal muscle and heart tissues [3]. The subsequent hyperinsulinemia inhibits hormone sensitive lipase and triggers the lipoprotein lipase causing additional glucose intolerance, hyperinsulinemia, hypertriglyceridemia and higher risk of insulin resistance in these tissues [4].

Analysis of complex biological systems has become possible by the newly emerging metabolomics techniques where metabolites serve as direct indicators of biochemical activity of complex phenotypes such as insulin resistance and T2DM [5]. In this context, lipidomics studies were utilized to study differences between SC and OM depots. These studies have revealed depot-specific enrichment of specific TAGs, glycerophospholipids, and sphingolipids and differences in the association of lipid species with body mass index, inflammation and insulin sensitivity [6, 7]. Although TAGs themselves are unlikely to be signaling molecules, an increasing body of evidence suggests that lipid

intermediates produced during TAG synthesis or breakdown interfere with the intracellular insulin signaling pathway and contribute to the development of insulin resistance, including free fatty acids, diacylglycerols and ceramides [8]. Indeed, elevated fatty acid efflux from the adipose tissue stimulates TAG synthesis in the liver and triggers stress of endoplasmic reticulum and stimulation of June kinase pathway in the adipose tissues [9, 10]. This leads to an overload of TAG's synthetic capacity, causing an increase in both diacylglycerols (DAGs) and ceramide levels and further development of insulin resistance in adipocytes [11].

Despite various studies investigating lipidomic differences in human serum and adipose tissues in relation to insulin sensitivity, no studies have compared differences in TAG signatures and their fatty acid composition in adipose tissues from IS, IR and T2DM obese individuals and their correlations with mediators of metabolic disease. Identification of the fatty acids that are enriched or depleted in tissues from insulin resistance and T2DM individuals could shed light on their functional role in disease progression, thus providing potential novel targets for therapeutic intervention. The aims of this study were to profile TAG species and measure their levels in two fat depots and to compare their fatty acid composition between IS, IR and T2DM individuals.

Methods

Materials: Interleukin 6 (IL-6) and leptin ELISAs were from R&D systems (Abingdon, UK). Insulin ELISA was from Mercodia Diagnostics (Uppsala, Sweden). 4',6-diamidino-2-phenylindole (DAPI), and LipidTOX Green Neutral Lipid were from Life Technologies (Warrington, UK). Other chemicals and reagents were from Sigma (Munich, Germany).

Cohort: Participants' recruitment criteria were described previously [12]. Briefly, 64 consented obese individuals undergoing bariatric surgery at AlEmadi hosptial (Doha, Qatar) were recruited. Protocols were approved by Institutional Review Board of ADLQ (X2017000224). Blood was taken prior to operation and 1–5 g of abdominal SC and OM adipose tissues biopsies were collected during the surgery and stored at -80^{o}C until use. Plasma cholesterol, fasting glucose and liver function enzymes were measured by COBAS INTEGRA (Roche Diagnostics, Basil). IL-6, leptin and insulin were determined using commercially available ELISA. Insulin resistance was computed by homeostatic model assessment (HOMA-IR) [13] using 30th percentile (HOMAIR= 2.4) as a threshold point. Accordingly, subjects were dichotomized into IS (HOMA-IR < 2.4, n=18, 3 Males and 15 Females) and IR (HOMA-IR > 2.4, n=35, 9 Males and 26 Females). Eleven participants were clinically diagnosed with T2DM (8 Males and 3 Females).

Preadipocyte culture and differentiation: Stromal vascular fraction (SVF) cells were obtained by collagenase digestion of adipose tissues as described previously [12]. Cell pellets were re-suspended in

stromal medium containing Dulbecco's modified Eagle's Medium-F12 (DMEM-F12) supplemented with 10% fetal bovine serum (FBS) and Penicillin/Streptomycin, then maintained at 37°C with 5% CO2 until confluence. To induce differentiation, early passaged stromal vascular fraction (SVF)-derived preadipocytes (passages 1–3) were grown at $2\times10^4/cm^2$ in stromal medium overnight, then incubated in differentiation medium (DMEM-F12, 3% FBS, 33 μM biotin, 17 μM D-pantothenate, 1 μM dexamethasone, 250 μM of methylisobutylxanthine, 0.1 μM human insulin, 5 μM of Peroxisome proliferator-activated receptor gamma PPARγ agonist, rosiglitazone) for 7 days, followed by 12 days in maintenance medium containing the same components as the differentiation medium but omitting methylisobutylxanthine and rosiglitazone. Differentiation potential (adipogenic capacity) was determined as a percentage of lipidtox positive stained cells to total number of stained nuclei (DAPI).

Sample preparation: Human SC and OM adipose tissue specimens from IS, IR and T2DM individuals were extracted using the Bligh& Dyer Method [14]. Homogenization of tissue was carried out in the gentleMACS Dissociator (Miltenyi Biotech, Germany) with one volume of PBS for every gram of tissue. Following tissue homogenization, 1 ml of each sample solution was transferred into a separate 15 ml Falcon tube, and 3 ml of 3:1 ratio of Chloroform: MeOH were added into each tube. One ml of PBS was added and samples were centrifuged at 3000 RPM for 20 min at room temperature. The organic layer (bottom layer) was carefully transferred into new 15 ml Falcon tubes and evaporated to dryness under a stream of high purity nitrogen. Samples were then

reconstituted with 1:1:1 mixture of Hexane, Isopropanol, Acetonitrile. Subsequently the extracts were analysed using data dependent full scan MS and MS/MS acquisition using the Thermo LTQ VelosPro dual cell linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

Sample analysis: Separation of TAGs was carried out using non-aqueous reverse phase UHPLC separation (NARP), on a Dionex Ultimate 3000 UHPLC system, using acetonitrile w/ 0.1% formic acid (eluent A), and isopropanol w/ 10 mM ammonium formate (eluent B) as the mobile phase. The column was a Phenomenex UHPLC C30 core shell, 150 mm x 2.1 mm and 2.7 µm particle size (Phenomenex Torrance CA, USA). Gradient conditions started with 5% B held for 2 minutes, then raised to 50%B at 30 minutes, held for 10 minutes and then reduced to 5%B at 45 minutes and held for further 5 minutes.

Mass Spectrometry: MS analyses were conducted using the Thermo LTQ VelosPro dual cell linear ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), acquiring both full scan MS and subsequent data dependent full scan MS/MS product ion spectra with wide band activation. Target parent ions were automatically selected from an inclusion list. The low resolution full scan analysis provides molecular parent masses (M+NH4+). These parent ion full scan MS/MS analysis provided further elucidation of possible structures represented in each lipid (fatty acid composition). Relative abundances of each identified TAG were estimated from the height values for each extracted ion current profile for parent masses of each compound (M+NH4+)

Separation by Equivalent Carbon Number: The above UHPLC conditions (NARP) provide separations of TAGs by their Equivalent Carbon Number (ECN). The ECN is calculated, from the total number of non-glycerol carbons in the TAG minus twice the number of the double bonds in the molecule (ECN=CN-2DB). NARP eluted the TAGs from lower to higher ECN with increasing percent B in the eluent. NARP-HPLC is commonly used for TAG separation because it works on both the chain and absolute height or area counts for each identified TAG. As some of the TAG may not show baseline resolution, the height counts were chosen to better represent the TAG.

Statistical analysis: All statistical analyses were carried out using R version 3.2.1 and SIMCA 13.0.1 software (Umetrics, Sweden). Variables with skewed distributions were log transformed or taken the square root of as appropriate to ensure normality. An initial PCA was conducted to identify components that explain large proportion of the TAG variance. A repeated measures linear model incorporating confounders**:** gender, age, BMI, PC1&PC2 (derived from earlier principle component analysis, PCA) and covariates**:** tissue and diabetic status (IS, IR, T2DM) was used to assess the differences in each TAG between the two tissues and amongst the insulin/diabetes groups. The model was based on repeated measures statistics since a TAG measurement from an individual was taken from two separate tissues: SC and OM. The model allows the individual inherent variation to be taken out of the total variance. Such enhanced modelling of the error structure increases the model's ability to detect significance of covariate effects. Nonetheless,

we have repeated the analysis using the standard linear model and confirmed the superiority of the repeated measures linear model counterpart. The linear model was sometimes used when fitting the repeated measures model was not possible due to missing data. False discovery rate (FDR) multiple testing correction was also performed on the differentially expressed TAG species identified between adipose tissues from IS, IR and T2DM individuals. Fatty acid enrichment amongst diabetes/tissue significant TAGs was assessed using the one tailed Wilcoxon sum of the ranks test on the list of metabolites that differed significantly between IS, IR and T2DM after correcting for covariates including gender, age, BMI, PC1&PC2. The analysis was based on assessing the likelihood of randomly observing a given fatty acid that often amongst highly ranked TAGs along the list of all TAGs ordered by p-value as follows: For each of the following contrasts: subcutaneous versus omental, IR versus IS, IR versus T2DM and IS versus T2DM, TAGs were ranked by their p-values and a given fatty acid mapped to the ranks of TAGs within which it is found. The analysis proceeds by assessing the likelihood of obtaining the observed sum of fatty acid identified ranks by chance. If the fatty acid is observed amongst the significant TAG at the top of the list, the sum of the ranks would be too small to be explained by chance alone; hence the null hypothesis is rejected in favor of enrichment. Enrichment hits failed to remain significant after FDR multiple testing correction but data was reported because of agreement with literature as elaborated in the discussion section. A similar test was used to assess enrichment in constituent fatty acid saturation levels.

Results

General characteristics of participants: Sixty four (44 females and 20 males) obese and morbidly obese (BMI= 43.1±7.5 Kg/m2) participants were recruited from amongst patients undergoing weight reduction surgery. Participants exhibited hyperleptinemia and hyperinsulinemia and were dichotomized into IS and IR groups based on their HOMA-IR index and into T2DM based on their medical records. Compared to BMI-matched IS and IR subjects, T2DM individuals were older and had higher circulating levels of TAG and lower leptin (Table 1). Compared to females, males had higher mean arterial blood pressure (MAP) (93.7 vs 84.7, $p<0.01$) and lower HDL (1.1 vs 1.5, $p=0.05$) and leptin (42.0 vs 67.3, $p<0.01$) (Table S1). IS males had lower HOMA-IR than their age and BMI-match IS females, whereas IR males had higher HOMA-IR than their age, but not BMI, matched females (Table S1). Compared to obese subjects (n=26), the morbidly obese participants (n=46) had significantly higher BMI, SBP, IL-6, FPG and HOMA-IR (**Table S2**).

Table 1. General characteristics of participants.

Variables	IS	IR	T2DM	P value				IS+IR	P value
	(N = 18)	(N = 35)	(N = 11)	ANOVA	IS vs IR	IS vs T2DM	IR vs T2DM	(N = 46)	IS+IR vs T2DM
Age (years)	32.09 (9.7)	30.26 (9.3)	43.57 (9.4)	0.000	0.739	0.017	0.003	30.9 (9.4)	0.001
BMI (kg m^{-2})	41.44 (7.0)	43.31 (6.9)	45.53 (9.6)	0.38	0.583	0.394	0.731	42.7 (6.9)	0.322
SBP (mmHg)	119.0 (13.8)	122.67 (15.7)	132.14 (11.6)	0.13	0.616	0.111	0.268	121.5 (15.1)	0.075
DBP (mmHg)	66.1 (9.8)	69.98 (12.7)	73.33 (7.0)	0.3	0.422	0.369	0.783	68.7 (11.9)	0.35
MAP	83.4 (8.1)	88.08 (12.4)	93.53 (8.9)	0.11	0.288	0.130	0.499	86.6 (11.3)	0.15
Cholesterol (mmol/L)	4.4 (0.9)	4.6 (1.2)	5.22 (0.9)	0.24	0.785	0.215	0.364	4.5 (1.1)	0.122
LDL (mmol/L)	2.73 (0.8)	2.91 (0.9)	3.1 (1.0)	0.55	0.693	0.593	0.855	2.8 (0.8)	0.466
HDL (mmol/L)	1.33 (0.4)	1.46 (0.9)	1.29 (0.3)	0.74	0.793	0.990	0.844	1.4 (0.8)	0.678
Triglyceride (mmol/L)	1.13 (0.6)	1.27 (0.7)	1.91 (1.1)	0.04	0.729	0.031	0.072	1.2 (0.6)	0.015
Leptin (ng/mL)	64.36 (25.5)	60.7 (21.9)	39.16 (31.5)	0.06	0.854	0.055	0.091	62.1 (23.1)	0.021
Adiponetin (ng/mL)	3.24 (2.2)	3.62 (1.9)	3.47 (2.4)	0.88	0.866	0.982	0.992	3.5 (1.9)	0.979
IL-6 (pg/mL)	3.28 (1.8)	3.72 (1.8)	4.03 (2.2)	0.58	0.683	0.627	0.913	3.6 (1.8)	0.521
FBG (mmol/L)	5.73 (2.5)	12.76 (8.4)	12.84 (6.5)	0.000	0.001	0.072	1.000	10.3 (7.7)	0.439
Insulin (mIU/L)	6.33 (1.9)	12.6 (10.0)	11.92 (6.4)	0.01	0.010	0.241	0.976	10.3 (8.6)	0.633
HOMA-IR	1.56 (0.6)	4.86 (2.0)	6.6 (3.2)	0.000	0.000	0.000	0.080	3.7 (2.3)	0.005

BMI (body mass index), SBP (systolic blood pressure), DBP (diastolic blood pressure), MAP (mean arterial blood pressure), LDL (low density lipoprotein), HDL (high density lipoprotein), IL-6 (interleukin 6), FPG (fasting blood glucose), HOMA-IR (homeostatic model assessment of insulin resistance). Data are presented as mean (SD). Differences between IS, IR and T2DM were tested by ANOVA. Differences between (IS+IR versus T2DM) were tested by the independent-sample t test or Mann–Whitney U test. A p-value significance level of 0.05 was used.

Differences in TAG content between omental and subcutaneous adipose tissues: Using a non-targeted approach, a comprehensive parent mass list of 120 identified TAGs was created, of which 76 TAG species were identified (appendix 1) based on their molecular weights and peak heights. A linear model was used to assess depot-specific TAG associations after correcting for participant diabetes group, gender, PC1 and PC2 (refer to methods). Analysis revealed 7 TAGs that

were significantly different between SC and OM tissues. C53:5, C51:3, C50:4, C59:1, C54:6 and C50:2 were higher in OM than SC. C38:1 was higher in the SC compared to OM tissues. The full scan MS/MS analysis revealed the fatty acid composition for each identified TAG (Table 2).

Table 2. Differential TAG species identified between subcutaneous and omental adipose tissues.

ID	TAG	MW	Fatty acid composition	Fatty acids identities	Fold change (SC-OM)	P value
TAG47	C53:5	866.7	C17:0, C17:1, C19:4	Heptadecanoic acid, *cis*-10-heptadecanoic acid, C19:4	0.17	0.01
TAG2A	C38:1	664.7	C18:1, C16:0, C4:0	Oleic acid, palmitic acid, butyric acid	– 0.44	0.02
TAG36	C51:3	842.6	C18:1, C16:1, C17:1	Oleic acid, palmitoleic acid, *cis*-10-heptadecenoic acid	0.32	0.03
TAG31	C50:4	826.7	C18:2, C18:2, C14:0	Linoleic acid, linoleic acid, myristic acid	0.37	0.03
TAG81	C59:1	958.8	C23:0, C18:0, C18:1	Tricosanoic acid, stearic acid, oleic acid	0.16	0.04
TAG53A	C54:6	878.7	C18:2, C18:2, C18:2	Linoleic acid, linoleic acid, linoleic acid	0.17	0.04
TAG33	C50:2	830.8	C18:2, C16:0, C16:0	Linoleic acid, palmitic acid, palmitic acid	0.19	0.05

Molecular weight (MW), fatty acid composition, fatty acid identity, fold change in SC tissue compared to OM are also indicated.

TAGs with varying levels between IS, IR and T2DM: A linear model was used to assess TAG associations with participant groups after correcting for possible confounders (refer to methods). A number of TAGs were significantly decreased in T2DM compared to IS and/or IR including C46:4, C48:5, C48:4, C38:1, C50:3 and C40:2 whereas a number of TAGs were increased in T2DM compared to the other two groups including C56:3, C56:4, C56:7 and C58:7. No significant differences in TAGs between IS and IR groups was detected. Table 3 summarizes the list of differentially expressed TAGs with their fatty acids compositions. When looking at gender vs group (IS, IR and T2DM) interaction, there

were no FDR significant interaction effects. However, when considering BMI vs group interaction, two TAG species showed FDR significant interaction effects including C40:2 and C53:4. Whereas the former (C40:1) shows more pronounced decrease in T2DM compared to IS in low BMI than in high BMI, the latter (C53:4) shows a more pronounced increase in low BMI than in high BMI (**Table S3**).

Table 3. Differentially expressed TAG species identified between adipose tissues from IS, IR and T2DM individuals.

ID	TAG	MW	Fatty acid composition	Fatty acids identities	Comparison	Fold change	FDR p value
TAG16A	C46:4	770.7	C18:2, C18:2, C10:0	Linoleic acid, linoleic acid, capric acid	IS vs T2DM	− 0.62	0.005
					IR vs T2DM	− 0.53	0.01
TAG21	C48:5	796.7	C18:2, C18:3, C12:0	Linoleic acid, linolenic acid, lauric acid	IR vs T2DM	− 0.39	0.0005
					IS vs T2DM	− 0.38	0.0013
TAG22	C48:4	798.7	C18:2, C18:2, C12:0	Linoleic acid, linoleic acid, lauric acid	IR vs T2DM	− 0.96	0.002
TAG2A	C38:1	664.7	C18:1, C16:0, C4:0	Oleic acid, palmitic acid, butyric acid	IS vs T2DM	− 1.00	0.0007
TAG32	C50:3	828.8	C16:1, C16:1, C18:1	Palmitoleic acid, palmitoleic acid, oleic acid	IR vs T2DM	− 0.78	1.37E−05
					IS vs T2DM	− 0.76	5.62E−05
TAG61	C56:7	904.8	C20:4, C18:1, C18:2	Arachidonic acid, oleic acid, linoleic acid	IS vs T2DM	0.81	0.0006
					IR vs T2DM	0.74	0.001
TAG64	C56:4	910.8	C18:1, C18:2, C20:1	Oleic acid, linoleic acid, gadoleic acid	IS and IR vs T2DM	0.98	0.004
TAG65	C56:3	912.8	C20:1, C18:1, C18:1	Gadoleic acid, oleic acid, oleic acid	IR vs T2DM	0.51	0.002
					IS vs T2DM	0.54	0.002
TAG7	C40:2	690.7	C6:0, C16:0, C18:2	Caproic acid, palmitic acid, linoleic acid	IS vs T2DM	− 1.07	1.26E−05
					IR vs T2DM	− 0.83	0.0002
TAG74	C58:7	934.8	C18:6, C24:0, C16:1	C18:6, lignoceric acid, palmitoleic acid	IS and IR vs T2DM	0.48	0.007
TAG75	C58:4	938.7	C18:3, C24:0, C16:1	Linolenic acid, lignoceric acid, palmitic acid	IR vs T2DM	0.69	0.0005
					IS vs T2DM	0.73	0.0005
TAG9	C42:2	718.7	C18:2, C12:0, C12:0	Linoleic acid, lauric acid, lauric acid	IS vs T2DM	− 0.77	0.0008
					IR vs T2DM	− 0.71	0.001

Molecular weight (MW), fatty acid composition, fatty acid identity, fold change between specified groups are also indicated.

An orthogonal partial least square discriminate analysis (OPLS-DA) comparing subjects from IS, IR and T2DM revealed two significant class-discriminatory components (R2X=0.18, R2Y=1, R2Q2=0.27, CV-ANOVA p value=0.0001) (Figure 1). The score plot in Figure 1A indicates an x-axis differentiating the T2DM group from IS and IR; the latter two groups being rather separated along the y-axis. The corresponding loading score, shown in Figure 1B, features similar TAG/group associations to those obtained with the linear model (shown in table 3). Specifically, lower amounts of C38:1, C46:4, C48:5 and C48:4 as opposed to higher levels of C58:7, C56:4, C56:4 and C56:7 in the T2DM group (also circled in red, **Figure 1B)**.

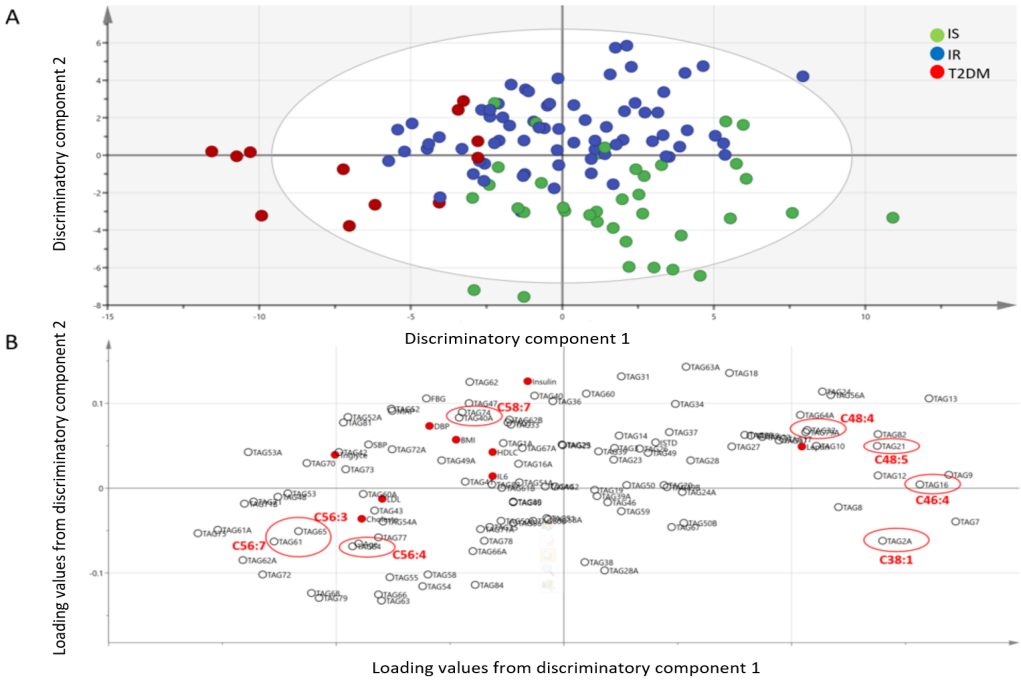

Figure 1. OPLS-DA model comparing adipose tissue-derived TAGs from IS, IR and T2DM individuals. (a) A score plot showing the class-discriminatory component 1 (*x*-axis) versus class-discriminatory component 2 (*y*-axis). (b) The corresponding loading plot showing similar TAG/ diabetes group associations to the linear model (circled in red).

In order to study the possible enrichment/depletion of certain fatty acid constituents of TAGs in association with insulin sensitivity or diabetes, an enrichment analysis based on the Wilcoxon sum of the ranks test was conducted (refer to methods). The results of the analysis are presented in Table 4 and further illustrated on **Figure 2.** Overall, C12:0 appears to be associated with TAGs least abundant in T2DM in both tissues whereas C18:3 is found in both depleted and enriched TAGs in T2DM (both sides of the x-axis in Figure 2). This could be justified by a potentially induced flow of C18:3 in certain recipient TAGs at the expense of other TAGs with diabetes. Further supporting this are the observed negative correlations between depleted and enriched C18:3 carrying TAGs (Figure 3). Interestingly, many of the C12:0 and C18:3 containing TAGs, including TAG21, TAG22, TAG75 and TAG9, were previously identified as significantly changing in level with diabetes by the linear model (**Table3)**.

Furthermore, C18:3 was also found to have a strong tissue signature featuring frequently amongst highly ranked TAGs from statistical analysis of TAG association with tissue type (data not shown). However, C18:3 does not feature amongst the TAGs found to significantly differ between tissues by the linear model (**Table 2**). This indicates that the collective tissue changes in C18:3 containing TAGs were rather subtle.

The Wilcoxon sum of the ranks analysis was also used to look for enrichment of fatty acid saturation level amongst the highly ranked significant TAGs from comparison of tissue/diabetes-insulin sensitivity groups but no significance was detected.

Table 4: TAG fatty acid association with tissue and diabetes/insulin sensitivity groups.

Compared groups	Fixed variable	Fatty acid	p value
IR × T2DM	SC	C12:0	0.045
	SC	C18:3	0.048
	OM	C12:0	0.016
	(Full model) SC+OM	C12:0	0.025
IS × T2DM	OM	C12:0	0.03
	OM	C18:3	0.048
SC × OM	(Full model) IS+IR+T2DM	C18.3	0.027

Analysis conducted using the Wilcoxon sum of the ranks test indicates fatty acids that were overrepresented amongst hit TAGs when comparing the groups specified in column 1. Comparing IS, IR and T2DM was done in individual tissues as well as when pooling data from the two tissues. Similarly, tissues were compared per group and when groups were combined (column 2). Only significant results are shown at a nominal p-value of 0.05.

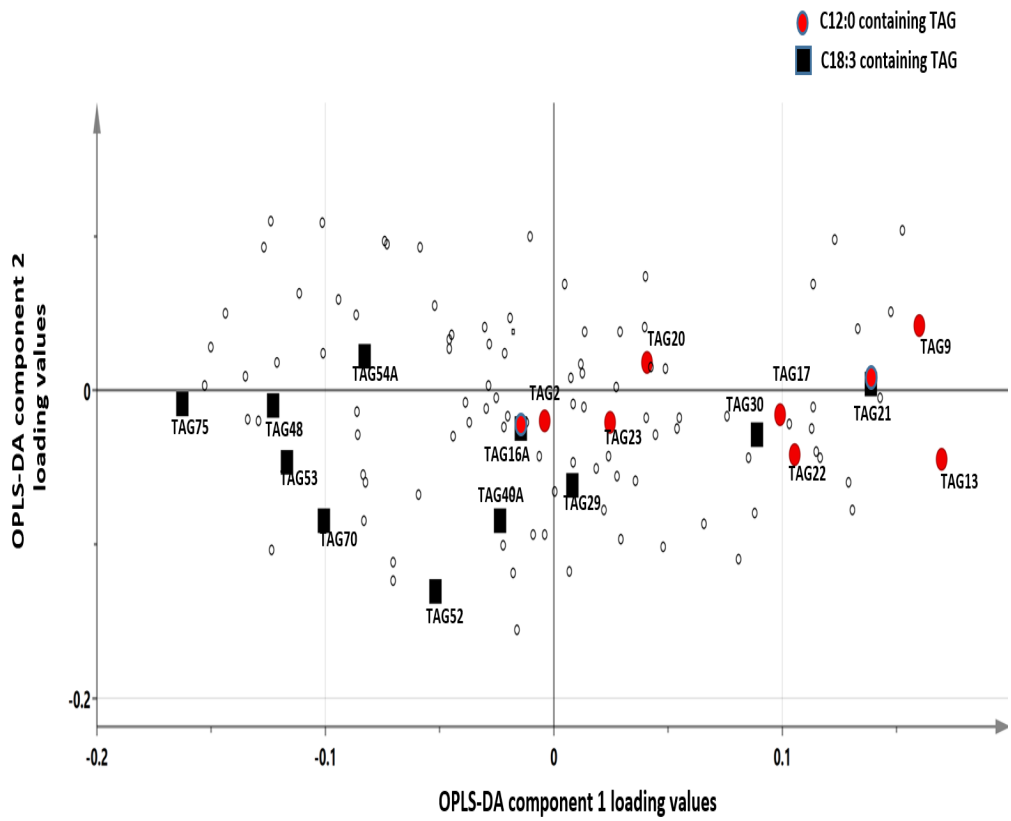

Figure 2: An OPLS-DA loading plot showing the spread of C12:0 and C18:3 containing TAG along the x-axis found previously (Figure 1A) to differentiate T2DM from IS+IR subjects**.** Unlike the C12:0 containing TAGs, the TAGs comprising C18:3 feature on both sides of the x-axis implying depletion of certain recipient TAGs (right side) as oppose to enrichment of others (left side) with diabetes.

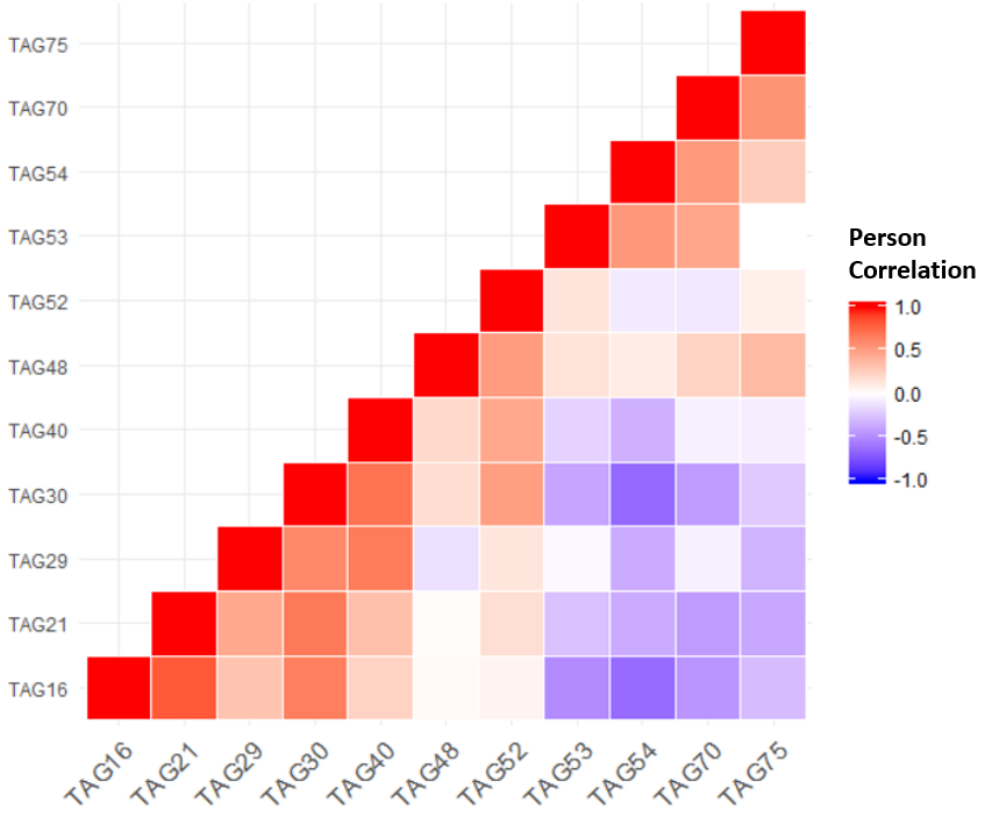

Figure 3. A triangular heatmap showing correlations between C18:3 carrying TAGs.

Correlation of TAG species with mediators of metabolic disease: A step-wise regression was performed to identify the best TAG predictors of various traits including age, BMI, systemic TAG, total cholesterol, IL-6 and HOMA-IR, SC and OM adipogenic capacity reported previously [12, 15] and shown in Figure S1. Table 5 lists TAG species identified with significant (p=0.0001) association with various metabolic traits and shows their importance and fatty acid compositions.

Table 5. List of TAGs associated with metabolic traits such as age, BMI, TC, TG, IL-6, HOMA-IR, SC and OM adipogenic capacity.

Metabolic trait	R^2	Importance	TAG	MW	Fatty acid composition	Fatty acids identities
Age	0.4	0.12	C56:1	916.8	C20:0, C18:0, C18:1	Arachidic acid, stearic acid, oleic acid
		0.12	C54:8	874.8	C18:3, C18:3, C18:2	Linolenic acid, linolenic acid, linoleic acid
BMI	0.5	0.12	C57:1	930.8	C17:0, C24:0, C16:1	Heptadecanoic acid, lignoceric acid, palmitoleic acid
		0.1	C48:1	804.8	C18:0, C16:1, C14:0	Stearic acid, palmitoleic acid, myristic acid
		0.09	C54:5	880.8	C18:1, C18:1, C18:3	Oleic acid, oleic acid, linolenic acid
TAG	0.5	0.12	C52:1	860.8	C16:0, C18:1, C18:0	Palmitic acid, oleic acid, stearic acid
		0.11	C54:1	888.8	C18:0, C18:0, C18:1	Stearic acid, stearic acid, oleic acid
TC	0.4	0.35	C40:2	690.7	C6:0, C16:0, C18:2	Caproic acid, palmitic acid, linoleic acid
IL-6	0.6	0.13	C38:1	664.7	C10:0, C12:0, C16:1	Capric acid, lauric acid, palmitoleic acid
		0.1	C42:1	720.7	C16:0, C16:1, C10:0	Palmitic acid, palmitoleic acid, capric acid
		0.07	C56:1	916.8	C18:0, C18:0, C20:1	Stearic acid, stearic acid, gadoleic
HOMA-IR	0.5	0.09	C44:2	746.7	C18:2, C14:0, C12:0	Linoleic acid, myristic acid, lauric acid
		0.09	C56:7	904.8	C20:4, C18:1, C18:2	Arachidonic acid, oleic acid, linoleic acid
SC adipogenic	0.9	0.16	C58:10	926.8	C18:2, C18:2, C22:6	Linoleic acid, linoleic acid, docosahexaenoic acid
		0.16	C56:4	910.8	C18:1, C18:2, C20:1	Oleic acid, linoleic acid, gadoleic acid
		0.14	C57:4	924.7	C22:0, C19:4, C16:0	Behenic acid, C19:4, palmitic acid
		0.09	C40:1	692.7	C18:1, C16:0, C6:0	Oleic acid, palmitic acid, caproic acid
		0.08	C60:1	970.8	C24:0, C24:0, C18:1	Lignoceric acid, lignocerric acid, oleic acid
		0.22	C38:1	664.7	C18:1, C16:0, C4:0	Oleic acid, palmitic acid, butyric acid
OM adipogenic	1	0.18	C48:1	804.8	C18:0, C16:1, C14:0	Stearic acid, palmitoleic acid, myristic acid
		0.14	C49:1	818.7	C18:1, C17:0, C14:0	Oleic acid, heptadecanoic acid, myristic acid
		0.11	C56:1	916.8	C18:0, C18:0, C20:1	Stearic acid, stearic acid, gadoleic
		0.09	C54:0	890.8	C18:0, C18:0, C18:0	Stearic acid, stearic acid, stearic acid,
		0.06	C38:0	666.7	C10:0, C14:0, C14:0	Capric acid, myristic acid, myristic acid
		0.05	C56:2	914.8	C18:1, C18:1, C20:0	Oleic acid, oleic acid, arachidic acid
		0.04	C51:1	846.7	C18:1, C15:0, C18:0	Oleic acid, pentadecanoic acid, stearic acid

A step-wise regression was performed to identify the best TAG predictors of various traits. A p-value significance level of 0.001 was used.

Discussion

TAGs constitute over 99% of lipid species in the adipose tissue of healthy individuals, with cholesterol and phospholipids making minor contributions [16]. TAGs are located within dynamic functional organelles known as lipid droplets that play important roles in intracellular vesicle trafficking, cell signaling and lipid homeostasis [17]. Although TAGs are not signaling molecules, fatty acids produced during their synthesis or breakdown were shown to interfere with the intracellular insulin signaling pathway and contribute to the development of insulin resistance [10]. Previous studies investigating TAG and fatty acid composition between subcutaneous and omental depots were published [6, 7]. However, this is the first study comparing TAGs and their fatty acid species in adipose tissues derived from IS, IR and T2DM obese individuals. Current technologies enable high-throughput profiling of the lipidome [18, 19]. In this study, LC/MS-based lipid profiling was performed to identify adipose signature of obesity-associated insulin sensitivity, insulin resistant and T2DM. The emerging data reveal differences in TAG species between SC and OM adipose tissues such as C38:1, C53:5, C51:3, C50:4, C59:1, C54:6 and C50:2 and among IS, IR and T2DM obese individuals including C46:4, C48:5, C48:4, C38:1, C50:3, C40:2, C56:3, C56:4, C56:7 and C58:7. The data also show differences in fatty acid compositions of TAGs associated with T2DM such as C12:0 and C18:3, suggesting a potential functional role of the identified species. Significant associations between the identified TAG species and traits of metabolic syndrome such as age, BMI, lipids (total cholesterol and circulating TAG), the inflammatory marker IL-6 and adipogenic capacity of preadipocytes derived from the same adipose tissues were

identified. These associations could shed light on the molecular mechanisms contributing to the increased risk of metabolic disease.

Depot specific differences: Our data identified few TAGs that were differently expressed between SC and OM tissues. One TAG that was higher in SC compared to OM was C38:1, which contains C4:0 (butyric acid). The latter was shown before to inhibit lipolysis and increase insulin sensitivity in primary rat adipocytes [20], perhaps contributing to the greater association of insulin resistance with OM mass compared to SC mass [21]. A previous study in obese men has shown increased C50:0, C59:2, C58:2, C60:3, C64:4, C51:0 and C65:1 fatty acids in OM compared to SC adipose tissues [6]. Changes in lipid composition between the two depots were attributed to differences in adipocyte differentiation, metabolism of the lipid droplet, and extent of beta-oxidation [6]. Differences between the two studies may reflect ethnic and/or diet differences between our Asian and the other study's Caucasian population. Variations in fatty acid composition between SC and OM fat depots confirm the specific metabolism of each depot, as selective lipolytic and lipogenic mechanisms may function in each tissue depot. Indeed, studies have shown that desaturase enzymes, regulating the number of saturated fatty acids, exhibit a depot-specific profile [22] in close association with insulin resistance [23].

IS, IR and T2DM specific differences: Systemic levels of fatty acid increase with obesity and T2DM, perhaps as a result of insulin resistance of adipose tissue and subsequent increased lipolysis; although in some obese individuals, fatty acid release from adipose tissues is

reduced per Kg fat in order to normalize plasma non-esterified fatty acid concentrations [24]. In our study, significant differences in levels of TAG composition were detected between IS, IR and T2DM. A number of candidates were either increased or decreased with risk of insulin resistance and T2DM, despite the predominant view of TAGs as an adverse risk factor for diabetes. Using the Wilcoxon sum of ranks statistics, fatty acids frequently occurring in highly-ranked TAGs along the list of TAGs ordered by p-value from diabetes association analysis were revealed. Two fatty acids were identified: C12:0 and C18:3. The strength of this enrichment analysis approach is that, unlike the Fishers' exact test, no arbitrary significance cut-off is applied on the list of TAGs. However, a possible weakness relates to the fact that since the TAGs are ordered by p-value, no account is given to the direction of change and therefore one may not speak of depletion or increase in fatty acid TAG level but rather a dynamic in metabolic activity involving the fatty acid in association with the phenotype of interest. This was observed with C18:3, and a negative correlation was noted between C18:3 host TAGs found increased and others decreased with diabetes, effectively suggesting a metabolic link between the two sets of TAGs. Our findings confirm previous studies that showed significant correlations of specific fatty acids with insulin sensitivity. These include a cross-sectional analysis of adipose tissue biopsies from elderly obese men, which identified positive correlations between levels of C12:0, C18:2 and C18:3 and insulin sensitivity [25]. Our data also confirmed the association of C18:3 with metabolic status as shown previously in two groups of obese individuals who underwent weight loss surgery [26]. Furthermore, subjects in the most insulin-sensitive quintile showed a significantly higher

percentage of circulating C18:2 (pre-cursor of C18:3) than the remaining subjects [27], further confirming our data. Functionally, previous work implicated C18:2 in the modulation of insulin signaling in rat skeletal muscle [28]. Therefore, our findings confirm previous results with regard to the association of C12:0 with insulin sensitivity [25], perhaps through triggering Glut4 translocation [27]. Our data also revealed reduction in C18:3 with T2DM incidents. This also confirms previous findings showing a negative correlation of C18:3 and its precursor with insulin resistance and positive association with insulin sensitivity [27].

Association of TAGs with mediators of metabolic syndrome: Further, our data highlight a panel of TAGs that were associated with mediators of metabolic disease in obese individuals. Increased age was associated with accumulation of C56:1 that is composed of saturated fatty acids C20:0 and C18:0 and mono-unsaturated C18:1, whereas age was negatively correlated with C54:8 that is composed of unsaturated fatty acids C18:2 and C18:3. Although participants had comparable BMI, the small increase in BMI was positively correlated with three unsaturated TAGs (C57:1, C48:1 and C54:5). Whereas circulating TAGs were associated with accumulation of C52:1 and C54:1 in the adipose tissue, total cholesterol was positively correlated with C40:2. The negative correlation between IL-6 and C38:1, C42:1 and C56:1 may suggest an anti-inflammatory effect of fatty acids that constitute these TAGs, in particular C10:0 that was shown previously to exert an anti-inflammatory properties [29]. HOMA-IR was also negatively correlated with C44:2 and C56:7, both containing C18:2 shown previously to negatively correlate with insulin resistance [25].

Association of TAGs with adipogenic capacity: Several TAGs were highly correlated with SC or OM adipogenic capacity. Previous studies have shown that the greater adipogenic capacity of SC and OM preadipocytes taken from IS obese individuals compared to IR and T2DM counterparts is partially mediated by lower IL-6 secretion and oxidative stress [12, 15, 30]. Secretion of interleukin IL-6 is significantly decreased after treatment with C18:2, C22:6 and C16:0 via inhibition of nuclear factor kappa B (NF-κB) and subsequent activation of the master regulator of adipogenesis, PPARγ [31]. Our data revealed positive correlations of C56:4 and C57:4, containing C18:2, C16:0, with SC adipogenic capacity. OM adipogenic capacity was associated with C49:1, C38:0 and C56:2, containing C16:0, C18:1 and C14:0. These fatty acids were shown previously to induce adipocyte differentiation in rodents [32-36] and potentially play a similar role in human preadipocytes.

Study limitations: One main limitation of this study is the relatively low number of participants, especially in the T2DM group. Additionally, the difference in gender distribution between IS and IR groups (predominantly females) and T2DM (predominantly males) group may have introduced bias in the study design that may have influenced the results. Despite these factors, clear TAG and fatty acid signatures were identified after correcting for potential confounders such as gender and BMI. Another limitation of the current work is its focus on association of TAGs with insulin resistance and risk of T2DM without an absolute quantitation of any specific analyte. Incorporating isotope-labeled standards would allow absolute quantitation and improve the precision

of measurements. Finally, differences in TAG composition in adipose tissues among the studied groups may have been influenced by their diet. Indeed, previous studies have shown that the process of fatty acid and TAG deposition in rat adipose tissue depends on the composition of the diet [37]. Dietary linoleic acid content was shown to influence the distribution of TAG species in rat adipose tissue, particularly di- and trilinoleoyl containing TAG as a result of linoleic acid intake [38]. Other studies have shown that the composition of TAG in rat epididymal, subcutaneous and perirenal adipose tissues was broadly reflecting dietary oils such as isomeric octadecenoic acids from coriander oil and high oleic sunflower oil [39]. Taken all these limitations into account, confirmation in different populations is warranted to validate these findings.

Conclusion

In summary, our data supports the dynamic nature of adipose tissue and the complex interaction between adipose tissue physiology and its lipid composition. The TAGs and their fatty acid composition within human adipose tissues from obese subjects are markedly different, depending on the insulin sensitivity status of the donors. Our data suggest that adipose tissues from obese IR and T2DM individuals exhibit TAG-specific signatures that may contribute to their increased risk compared to their insulin-sensitive counterparts or could reflect different dietary consumption among the studied groups. Future experiments are warranted to investigate the functional relevance of these specific lipidomic profiles with reference to participants' consumed diet.

List of abbreviations

4',6-diamidino-2-phenylindole (DAPI)
Body mass index (BMI)
Diastolic blood pressure (DBP)
Diacylglycerols (DAGs)
Effective carbon number (ECN)
Fasting blood glucose (FPG),
High density lipoprotein (HDL)
Homeostatic model assessment (HOMA-IR)
Insulin sensitive (IS)
Insulin resistant (IR)
Interleukin 6 (IL-6)
LDL (low density lipoprotein),
MAP (mean arterial blood pressure),
Non-aqueous reverse phase UHPLC separation (NARP)
Nuclear factor kappa B (NF-κB)
Omental (OM)
Orthogonal partial least square discriminate analysis (OPLS-DA)
Principle component analysis (PCA)
Retention time (RT)
Systolic blood pressure (SBP)
Stromal vascular fraction (SVF)
Subcutaneous (SC)
Triacylglycerols (TAGs)
Type 2 diabetes mellitus (T2DM)
Ultra-high performance liquid chromatography (UHPLC)

Declarations

Ethics approval and consent to participate

All participants were consented. Protocols were approved by Institutional Review Board of ADLQ (X2017000224).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

HS carried out most of the data acquisition and analysis, helped with drafting the article and approved the final version. ID carried out the statistical analysis and helped with data interpretation. SB helped with data acquisition. ME and PA contributed to the study design and provided samples and information on participants. TMH helped with processing and interpretation of LC-MS-MS data. AD contributed to HS's supervision and advised on experimental design. AL conceived the idea and contributed to experimental design and data interpretation. MAE was lead principle investigator, designed the experiments, supervised progress, analysed data and wrote and approved the final version of the article. MAE is responsible for the integrity of the work as a whole.

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Not applicable

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Table S1. Comparison of participants' characteristics by gender.

Variables	Gender	All (44F, 20M)			IS (14F, 4M)			IR (24F, 11M)			T2DM (6F, 5M)		
		Mean	SD	P value	Mean	SD	P value	Mean	SD	P value	Mean	SD	P value
Age (year)	Female	31.5	9.3	0.408	32.4	8.2	0.780	29.5	9	0.430	41	12.2	0.450
	Male	33.6	11.6		31	15.3		32	10.1		47	2.6	
BMI (kg.m-2)	Female	42.1	7.2	0.110	41.5	7.8	0.920	41.5	5.9	0.010	49.4	11.3	0.260
	Male	45.0	6.7		41.1	3.6		47.1	7.3		40.4	4.4	
SBP (mmHg)	Female	119.8	13.9	0.008	119	15	0.980	117.8	13.3	0.000	133.5	11.3	0.750
	Male	129.6	15.0		118.8	10.3		132.8	16.1		130.3	14.2	
DBP (mmHg)	Female	66.6	9.1	0.001	66.6	9.6	0.670	64.6	8.1	0.000	73.8	8.2	0.860
	Male	76.0	13.8		64.4	11.1		80.6	13.7		72.5	6.4	
MAP	Female	84.7	8.8	0.001	83.7	7.5	0.780	82.7	8.2	0.000	95.2	9.5	0.570
	Male	93.7	13.1		82.5	10.6		98	12.8		90.2	9.7	
Cholesterol (mmol/L)	Female	4.6	1.2	0.466	4.4	1	0.710	4.5	1.4	0.470	5	1.1	0.460
	Male	4.8	0.8		4.3	0.5		4.8	0.9		5.5	0.4	
LDL (mmol/L)	Female	2.8	0.8	0.169	2.7	0.9	0.880	2.8	0.8	0.190	2.9	1.3	0.590
	Male	3.1	0.8		2.7	0.3		3.2	0.9		3.4	0.8	
HDL (mmol/L)	Female	1.5	0.8	0.048	1.4	0.3	0.310	1.6	1.1	0.100	1.4	0.2	0.280
	Male	1.1	0.3		1.2	0.4		1.1	0.3		1.2	0.3	
Triglyceride (mmol/L)	Female	1.3	1.0	0.490	1.2	0.7	0.480	1.2	0.6	0.170	1.5	0.6	0.330
	Male	1.5	0.8		1	0.3		1.5	0.7		2.4	1.5	
Leptin (ng/ml)	Female	67.3	24.1	0.000	70.1	25.2	0.040	67.4	22.1	0.010	55.6	33.4	0.110
	Male	42.0	16.9		41.5	8		48.4	16.1		17.2	5.6	
IL-6 (pg/ml)	Female	3.7	2.0	0.594	3.5	1.9	0.260	3.6	1.9	0.690	4.7	2.7	0.400
	Male	3.4	1.5		2.3	0.9		3.9	1.7		3.2	0.3	
FBG (mmol/L)	Female	9.9	6.5	0.259	6.3	2.2	0.050	11.3	7.3	0.110	15.5	6	0.170
	Male	12.1	9.8		3.8	2.8		15.7	10		7.4	4.2	
Insulin (mIU/L)	Female	9.9	6.7	0.554	6.7	1.9	0.080	12.2	8.2	0.750	10	4.4	0.410
	Male	11.2	11.2		5	1.2		13.3	13.4		14.5	8.7	
HOMA-IR	Female	3.6	2.1	0.115	1.8	0.4	0.000	4.4	1.6	0.040	6.5	2.8	0.930
	Male	4.7	3.3		0.8	0.7		5.8	2.5		6.8	5.4	

Table S2. Differences between obese and morbidly obese subjects.

Obese (n=26), Morbidly Obese (n=40)		Mean	Std. Deviation	P value
Age	Obese	30.3	9.1	0.166
	Morbidly obese	33.7	10.4	
BMI	Obese	36.8	2.8	0.000
	Morbidly obese	46.8	6.2	
SBP	Obese	117.9	13.7	0.033
	Morbidly obese	125.6	15.1	
DBP	Obese	67.2	5.8	0.260
	Morbidly obese	70.5	13.6	
MAP	Obese	84.2	7.0	0.071
	Morbidly obese	89.3	12.6	
Cholesterol	Obese	4.7	1.1	0.675
	Morbidly obese	4.6	1.2	
LDL	Obese	2.9	0.7	0.856
	Morbidly obese	2.9	0.9	
HDLC	Obese	1.3	0.3	0.758
	Morbidly obese	1.4	0.9	
Triglyceride	Obese	1.2	0.6	0.118
	Morbidly obese	1.5	1.1	
Leptin	Obese	58.9	27.2	0.899
	Morbidly obese	59.7	24.0	
Adiponectin	Obese	3.8	2.2	0.393
	Morbidly obese	3.2	1.6	
IL6	Obese	2.7	1.6	0.005
	Morbidly obese	4.1	1.8	
FBG	Obese	7.9	5.7	0.025
	Morbidly obese	12.1	8.3	
Insulin	Obese	10.5	6.0	0.911
	Morbidly obese	10.3	9.6	
HOMA	Obese	3.1	1.6	0.034
	Morbidly obese	4.5	2.9	

Table S3. TAGs exhibiting BMI interaction.

ID	TAG	Fatty Acid Composition	Fatty Acids Identities	BMI	Fold Change	Std.Error	p-value	FDR p-value
TAG48	C53:4	Stearic acid, Linolenic acid, Cis-10-Heptadecanoic acid	IS vs T2DM	low	0.78	0.20	0.0003	0.04
			IS vs IR	low	0.12	0.12	0.35	0.71
			IS vs T2DM	high	0.16	0.18	0.36	0.73
			IS vs IR	high	0.01	0.10	0.91	0.98
TAG7	C40:2	Caproic acid ,palmitic acid, linoleic acid	IS vs T2DM	low	-1.61	0.32	4.42167E-06	0.002
			IS vs IR	low	-0.58	0.21	0.008	0.16
			IS vs T2DM	high	-0.69	0.29	0.02	0.25
			IS vs IR	high	-0.03	0.17	0.82	0.95

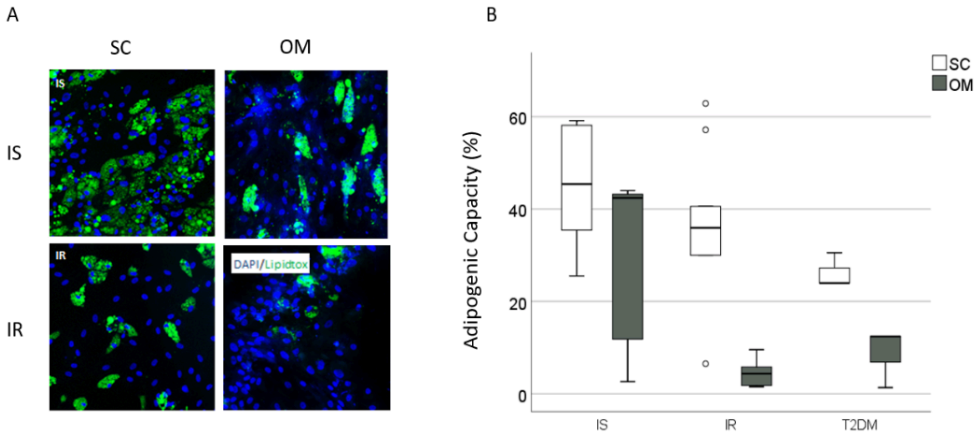
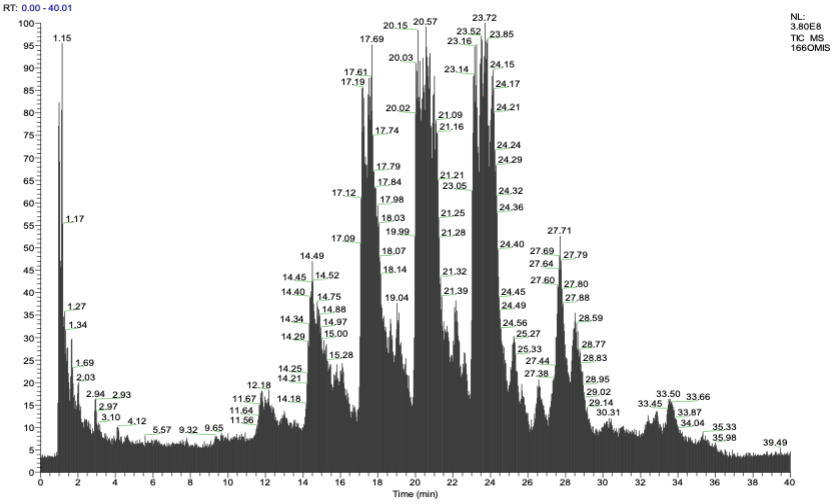


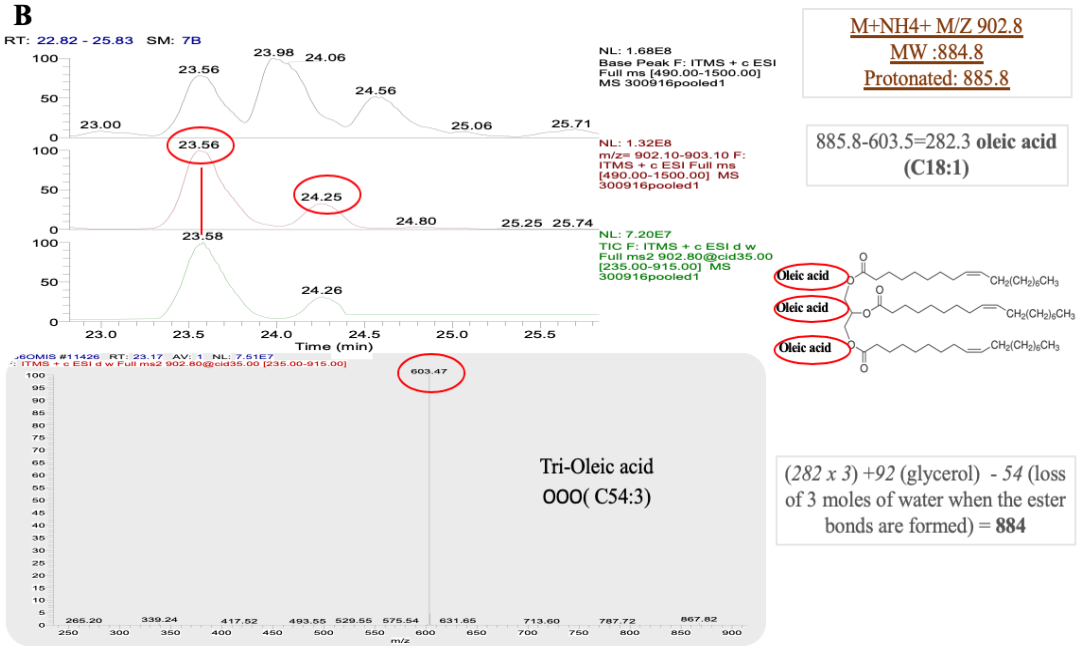
Figure S1. Adipogenic capacity of preadipocytes derived from subcutaneous (SC) and omental (OM) adipose tissues from insulin sensitive (IS), insulin resistant (IR) and type 2 diabetes mellitus (T2DM) patients. Representative images of SC and OM adipocytes form IS and IR individuals stained with DAPI in blue (nuclear staining) and lipidtox in green (lipid droplet staining) (A). A bar chart showing differences in the adipogenic capacity (percentage of differentiated adipocytes to total number of nuclei) in SC and OM preadipocytes derived from IS, IR and T2DM individuals (B). Significant differences in adipogenic capacity with disease progression were detected as reported previously [12, 15].

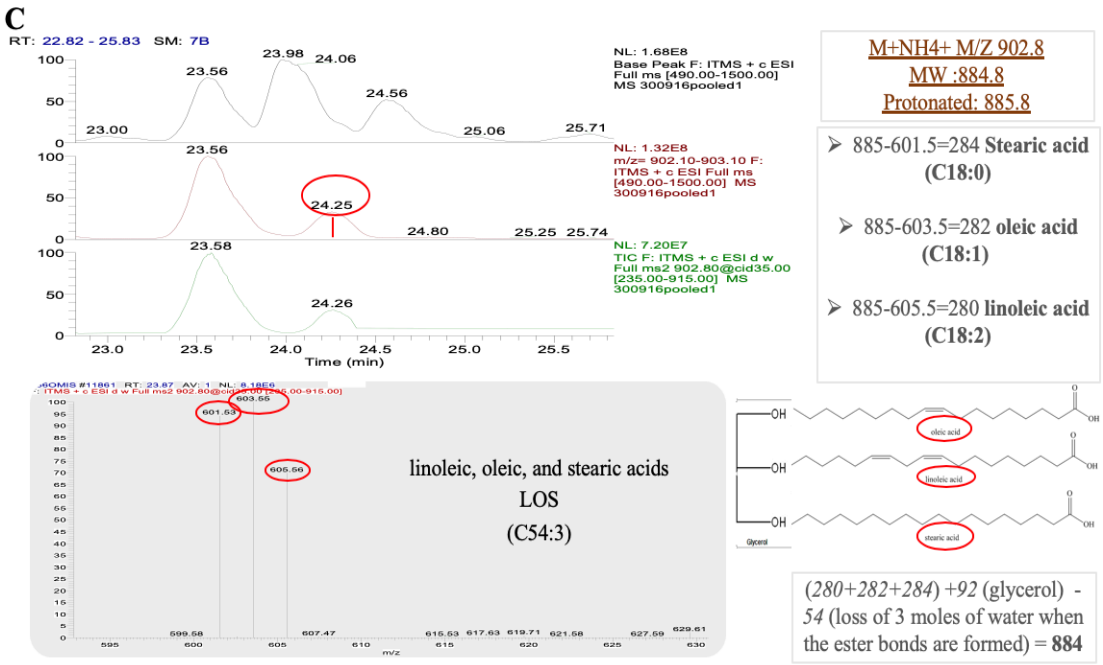
A



Using a nontargeted approach, a comprehensive parent mass list for 148 identified TAG was created including their molecular weights and peak heights

B





Supplementary data : Identification of Triacylglycerols (TAGs).
(A) Total Ion Chromatogram(TIC). **(B)** Example of Identification of TAG C54:3 at RT 23.56 minutes. **(C)** same TAG analyses on RT 24.25 minutes

Adipose Tissue Oil - NARP ESI dd MSMS

Peak #	Parent M+NH4	TAG MW	Comp	FA Comp	Height XIC x 10e3	MSMS Confirm	% Comp (H)
1	**842.8**	824.8	C50:5	LLC14:1	1853	linoleic,linoleic,C14:1	**0.158%**
2	**894.8**	876.8	C54:7	LLLn	1748	linoleic,linoleic,linolenic	**0.149%**
3	**868.8**	850.8	C52:6	LnLC16:1	1660	linoleic, linolenic,C16:1	**0.142%**
4	**816.8**	798.8	C48:4	LPoC14:1	1847	linoleic, palmitoleic, C14:1	**0.158%**
5	**894.8**	876.8	C54:7	OLC18:4	782	oleic,linoleic,C18:4	**0.067 %**
6	**816.8**	798.8	C48:4	LLC12:0	3901	Linoleic,linoleic,lauric	**0.333 %**
7	**790.8**	772.8	C46:3	LPoC12:0	1853	linoleic,palmitoleic,lauric	**0.158%**
8	**816.8**	798.8	C48:4	C12:1LO	619	linoleic,oleic,C12:1	**0.053 %**
9	**920.8**	902.8	C56:8	LLC20:4	3373	linoleic,lioleic,C20:4	**0.288 %**
10A	**790.8**	772.8	C46:3	OPoC12:1	690	oleic,palmitoleic,C12:1	**0.059 %**
10B	**790.8**	772.8	C46:3	MLC14:1		myristic,linoleic,C14:1	**0.000 %**
11	**894.8**	876.8	C54:7	PoLC20:4	3725	palmitoleic, linoleic, C20:4	**0.318%**
12	**868.8**	850.8	C52:6	C20:4C16:1C16:1	1149	C20:4,C16:1,C16:1	**0.098 %**
13A	**920.8**	902.8	C56:8	OLC20:5	1521	oleic,linoleic,C20:5	**0.130%**
13B	**920.8**	902.8	C56:8	PoLC22:5		palmitoleic,linoleic,C22:5	**0.000 %**
14	**790.8**	772.8	C46:3	LPC12:1	296	linoleic,palmitic,C12:1	**0.025 %**
15	**868.8**	850.8	C52:6	C14:0C20:4L	1008	myristic, linoleic,C20:4	**0.086 %**
16	**946.8**	928.8	C58:8	C22:5OL	1117	oleic,linoleic,EPA	**0.095 %**
17	**920.8**	902.8	C56:8	OPoC22:5	583	oleic,palmitoleic,DHA	**0.050 %**
18	**896.8**	878.8	C54:6	LLL	16809	linoleic, linoleic, linoleic	**1.437%**
19	**870.8**	852.8	C52:5	LLPo	19571	linoleic, linoleic, palmitoleic	**1.673%**
20A	**844.8**	826.8	C50:4	PoPoL	12782	palmitoleic,palmitoleic, linoleic	**1.092%**
20B	**844.8**	826.8	C50:4	LOC14:1		linoleic,oleic,C14:1	**0.000 %**

21	**896.8**	878.8	C54:6	OLLn	6378	oleic, linoleic, linolenic	**0.545 %**
22	**818.8**	800.8	C48:3	PoOC14:1	3583	palmitoleic,oleic,C14:1	**0.306 %**
23	**844.8**	826.8	C50:4	LLM	10929	linoleic,linoleic,myristic	**0.934 %**
24A	**818.8**	800.8	C48:3	OLC12:0	2268	lauric,oleic, linoleic	**0.194%**
24B	**818.8**	800.8	C48:3	LPoM		linoleic,palmitoleic,myristic	**0.000 %**
25	**922.8**	904.8	C56:7	C20:4LO	11301	oleic, linoleic,C20:4	**0.966 %**
26	**818.8**	800.8	C48:3	LPC14:1	7751	linoleic,palmitic,C14:1	**0.662 %**
27	**896.8**	878.8	C54:6	OPoC20:4	6618	oleic,palmitoleic,C20:4	**0.566 %**
28	**922.8**	904.8	C56:7	C22:5PL	3865	linoleic,palmitic,C22:5	**0.330 %**
29	**844.8**	826.8	C50:4	PoPLn	1771	palmitoleic,palmitic,linolenic	**0.151%**
30	**870.8**	852.8	C52:5	PLLn	2711	palmitic, linoleic, linolenic	**0.232 %**
31A	**792.8**	774.8	C46:2	LPC12:0	2152	linoleic, palmitic, lauric	**0.184%**
31B	**792.8**	774.8	C46:2	LMM		linoleic,myristic,myristic	**0.000 %**
32	**896.8**	878.8	C54:6	LPC20:4	13032	linoleic,palmitic,C20:4	**1.114%**
33	**870.8**	852.8	C52:5	PPoC20:4	6561	palmitic,palmitoleic,C20:4	**0.561%**
34	**898.8**	880.8	C54:5	OLL	60995	oleic, linoleic, linoleic	**5.213%**
35	**872.8**	854.8	C52:4	OLPo	53249	oleic, linoleic, palmitoleic	**4.551%**
36	**898.8**	880.8	C54:5	OOLn	5546	oleic, oleic, linolenic	**0.474 %**
37	**846.8**	828.8	C50:3	OPoPo	19049	oleic,palmitoleic,palmitoleic	**1.628%**
38	**872.8**	854.8	C52:4	LLP	56742	linoleic, linoleic, palmitic	**4.849 %**
39	**846.8**	828.8	C50:3	LPPo	8909	linoleic, palmitic, palmitoleic	**0.761%**
40A	**820.8**	802.8	C48:2	PPoPo	14709	palmitic,palmitoleic,palmitoleic	**1.257%**
40B	**820.8**	802.8	C48:2	OPC14:1		oleic,palmitic,C14:1	**0.000 %**
41	**860.6**	842.6	C51.3	OPoC17:1	3633	oleic, palmitoleic,C17:1	**0.310%**
42	**872.8**	854.8	C52:4	OLnP	3234	oleic,linolenic,palmitic	**0.276 %**
43	**820.8**	802.8	C48:2	LPM	6810	linoleic, palmitic, myristic	**0.582 %**
44	**794.8**	776.8	C46:1	OPC12:0	3304	oleic, palmitic, lauric	**0.282 %**

45	**898.8**	880.8	C54:5	POC20:4	16281	palmitic,oleic,C20:4	**1.391%**
46	**820.8**	802.8	C48:2	MLP	9003	myristic,linoleic,palmitic	**0.769 %**
47A	**926.8**	908.8	C56:5	LLC20:1	6969	linoleic,linoleic,C20:1	**0.596 %**
47B	**926.8**	908.8	C56:5	LOC20;2		linoleic, oleic, C20:2	**0.000 %**
48A	**860.8**	842.8	C51:3	LPC17:1	7685	linoleic, palmitic, C17:1	**0.657%**
48B	**860.8**	842.8	C51:3	OLC15:0		oleic, linoleic, C15:0	**0.000 %**
49	**900.8**	882.8	C54:4	OOL	92028	oleic, oleic, linoleic	**7.865%**
50	**874.8**	856.8	C52:3	OOPo	57486	oleic, oleic, palmitoleic	**4.913%**
51	**900.8**	882.8	C54:4	LPC20:2	10149	linoleic. Palmitic, C20:2	**0.867 %**
52	**874.8**	856.8	C52:3	OLP	100751	oleic, linoleic, palmitic	**8.611%**
53	**848.8**	830.8	C50:2	OPPo	75019	oleic, palmitic, palmitoleic	**6.412%**
54	**848.8**	830.8	C50:2	LPP	20034	linoleic, palmitic, palmitic	**1.712%**
55	**928.8**	910.8	C56:4	OOC20:2	10718	oleic,oleic,C20:2	**0.916%**
56	**822.8**	804.8	C48:1	OPM	21304	oleic, palmitic, myristic	**1.821%**
57	**902.8**	884.8	C54:3	OOO	86673	oleic, oleic, oleic	**7.408 %**
58	**902.8**	884.8	C54:3	OPC20:2	16632	oleic, palmitic, C20:2	**1.421%**
59	**902.8**	884.8	C54:3	SOL	12570	stearic, oleic, linoleic	**1.074%**
60	**876.8**	858.8	C52:2	OOP	112788	oleic, oleic, palmitic	**9.639 %**
61	**876.8**	858.8	C52:2	OPO	5656	oleic,palmitic,oleic	**0.483 %**
62	**876.8**	858.8	C52:2	LSP	6291	linoleic,stearic,palmitic	**0.538 %**
63	**850.8**	832.8	C50:1	OPP	24213	oleic, palmitic, palmitic	**2.069 %**
64	**930.8**	912.8	C56:3	SOC20:2	9512	leic, stearic,C20:2	**0.813%**
65	**850.8**	832.8	C50:1	POP	25049	oleic, palmitic, palmitic	**2.141%**
66	**930.8**	912.8	C56:3	OOC20:1	722	oleic,oleic,C20:1	**0.062 %**
67	**904.8**	886.8	C54:2	OPC20:1	8704	oleic, palmitic, gadoleic	**0.744 %**
68	**930.8**	912.8	C56:3	OSC20:2	6953	oleic,stearic,C20:2	**0.594 %**
69	**904.8**	886.8	C54:2	OOS	26940	oleic, oleic, stearic	**2.302 %**
70	**904.8**	886.8	C54:2	OSO	374	oleic,stearic,oleic	**0.032 %**

71	**904.8**	886.8	C54:2	ALP	387	arachidic,linoleic,palmitic	**0.033 %**
72	**878.8**	860.8	C52:1	SOP	8607	stearic, oleic, palmitic	**0.736%**
73	**878.8**	860.8	C52:1	OSP	2396	stearic oleic palmitic	**0.205 %**
74	**932.8**	914.8	C56:2	OOA	626	oleic,oleic,arachidic	**0.054 %**
75A	**824.8**	806.8	C48:0	SPM	343	Stearic, palmitic, myristic	**0.029 %**
75B	**824.8**	806.8	C48:0	PPP		palmitic, palmitic, palmitic	**0.000 %**
76	**906.8**	888.8	C54:1	SSO	473	stearic, stearic, oleic	**0.040 %**

Chapter 5: Association of Polybrominated Diphenyl Ethers in Two Fat Compartments with Increased Risk of Insulin Resistance in Obese Individuals

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Abstract

Background: Polybrominated diphenyl ethers (PBDEs), a widely utilized class of flame retardants in various commercial products, represent a prominent source of environmental contaminants. PBDEs tend to accumulate in adipose tissue, potentially altering the function of this endocrine organ and increasing risk of insulin resistance. The aim of this study was to compare levels of PBDEs in adipose tissues from two metabolically distinct obese groups; the insulin sensitive (IS) and the insulin resistant (IR).

Methods: Levels of 28 PBDE congeners were assessed in subcutaneous and omental adipose tissues from 34 obese Qatari individuals (11 IS and 23 IR) using gas chromatography (Trace GC Ultra) coupled to a TSQ Quantum triple Quadrupole mass spectrometer. Correlations of identified PBDEs and mediators of metabolic disease were established and effects of PBDEs treatment on insulin signaling in primary omental preadipocytes were determined.

Results: Out of 22 detectable PBDEs in subcutaneous and omental adipose tissues, PBDEs 28, 47, 99 and 153 were predominant in omental adipose tissues from obese Qatari subjects. PBDEs 99, 28, and 47 were significantly higher in IR individuals compared to their IS counterparts. Significant positive correlations were identified between PBDEs 28 and 99 in the omental tissues and with fasting insulin levels. When considering PBDEs congeners, penta congeners were also higher in IR compared to IS individuals, while no significant differences were detected in mono, tri, tertra, hexa, hepta and octa congeners between the two studied groups. Treatment of human omental preadipocytes from insulin sensitive individuals with PBDE28 caused inhibition of phosphorylation of GSK3 α/β (Ser^{21}/Ser^{9}), mTOR (Ser^{2448}), p70 S6 kinase (Thr^{389}) and S6 ribosomal protein (Ser^{235}/Ser^{236}) and activation of PTEN (Ser^{380}) phosphorylation, suggesting inhibition of insulin signaling.

Conclusion: This pilot data suggests that accumulation of specific PBDEs in human adipose tissues is associated with insulin resistance in obese individuals. Further investigation of the functional role of PBDEs in the pathology of insulin resistance should help developing therapeutic strategies targeting obese individuals at higher risk.

Keywords: Polybrominated diphenyl ethers, Endocrine-disrupting compounds, Adipose tissue, Insulin sensitivity, Insulin resistance, Obesity.

Abbreviations: Accelerated Solvent Extraction (ASE), An orthogonal partial least squares discriminant analysis (OPLS-DA), Body mass index (BMI), Diastolic blood pressure (DBP), Dichloromethane (DCM), Fasting blood glucose (FPG), Fetal bovine serum (FBS), High density lipoprotein (HDL), Homeostatic model assessment (HOMA-IR), Homeostatic model assessment of insulin resistance (HOMA-IR), Insulin receptor substrate 1 (IRS-1), Insulin resistant (IR, Insulin sensitive (IS), Interleukin 6 (IL-6), Low density lipoprotein (LDL), Mammalian target of rapamycin (mTOR), Mean arterial blood pressure (MAP), Multiple reaction monitoring (MRM) Omental (OM) Persistent organic pollutants (POPs), Phosphatase and tensin homolog (PTEN), Polybrominated diphenyl ethers (PBDEs), Polybrominated diphenyl ethers (PBDEs), Pressurized liquid extraction (PLE), Programmable temperature vaporization (PVT), Stromal vascular fraction (SVF), Subcutaneous (SC), Systolic blood pressure (SBP), Type 2 diabetes mellitus (T2DM).

1. Introduction

Obesity constitutes a major risk factor for cardiovascular disease, hypertension, cancer and type 2 diabetes mellitus (T2DM) [1]. However, a subset of obese individuals, known as the insulin sensitive (IS) or metabolically healthy obese (MHO), exhibit less metabolic deregulations compared to their obese insulin resistant (IR) counterparts [2]. The protected IS individuals maintain insulin sensitivity [2, 3] and show lower systemic levels of lipids and inflammatory markers [4]. During obesity, excess fat accumulates in the adipose tissues within the subcutaneous (SC) and visceral, including the omental (OM), fat compartments. This is often associated with ectopic fat deposition in the liver, skeletal muscle and heart tissues, leading to enhanced risk of insulin resistance and T2DM [9]. Whereas obesity-associated inflammation and oxidative stress were suggested to play a role in the increased risk of obese IR individuals compared to their IS counterparts [5-7], the potential role of endocrine-disrupting environmental pollutants in air, water and food in the increased risk of IR-obesity compared to IS-obesity has not yet been investigated.

Evidence linking persistent organic pollutants (POPs) accumulation and development of diabetes was previously described [8]. Associations of blood concentrations of various classes of POPs with increased prevalence of diabetes was shown in different populations through increasing risk of insulin resistance [9, 10]. Among the studied POPs, polybrominated diphenyl ethers (PBDEs) represent a class of flame retardants that were widely utilized in various commercial products [11]. Two hundred and nine known PBDE congeners that vary by the extent of

halogenations can leak freely into the environment [12, 13]. Despite cessation of their manufacturing since 2004, concerns of their bioaccumulation remain [14-16] due to their high stability in products manufactured before the ban and recycled materials [11] as well as their high intake during infancy [17]. Deca-BDE were only withdrawn from the N. American market by 2013 as their dehalogenation in environment generates lower-brominated PBDEs. Despite the success of policies in lowering the exposure to some PBDE congeners by eliminating their sources from the markets, exposures continue to rise in North America and may remain abundant in human populations [18, 19].

Presence of PBDEs in numerous products of daily use increases exposure at home environment [20]. PBDEs tend to accumulate in adipose tissue owing to their highly lipophilic nature [21]. Levels of PBDEs in obese individuals were positively correlated with visceral fat and visceral/subcutaneous abdominal fat ratio [22]. The bioaccumulation of PBDEs within adipocytes potentially alters their function by increasing lipolysis and decreasing glucose oxidation, causing increased risk of metabolic disease including obesity, insulin resistance and T2DM. Indeed, daily exposure to PBDE71 induced markers of insulin resistance including enhanced lipolysis and reduced glucose oxidation in rat adipocytes [23]. The maternal exposure of rodents to PBDE47 predisposed the offspring to increased body weight during early postnatal development and risk of metabolic dysfunction [24, 25], whereas PBDE47 exposure during the early postnatal period induced a mild disturbance in glucose metabolism in mice with increased baseline insulin sensitivity [26]. It also triggered significant transcriptomic changes in their

gonadal adipose tissue, placing fat tissue as a primary target for PBDE-47 [27]. This study also builds a background for a targeted search of sensitive phenotypic endpoints of BDE-47 exposure, including lipid profile parameters and coagulation factors in circulation. A positive correlation between serum levels of the PBDE153, metabolic syndrome and visceral fat mass in humans was previously established [28]. Furthermore, adipogenesis of mouse preadipoctyes was shown to increase in the presence of PBDEs in the absence of glucocorticoids, suggesting a different molecular target than the glucocorticoid receptor [29]. The versatility of PBDEs function on adipose tissue function, therefore, warrant further investigation.

Despite various studies investigating the association of serum PBDEs with metabolic disease and their potential function on adipocytes, no study has investigated the association of PBDEs within human adipose tissues from IS and IR obese individuals. In this study, we hypothesized that levels of certain PBDEs in human adipose tissues will be associated with incidents of insulin resistance in obese subjects and that PBDEs treatment of cells derived from IS adipose tissues could trigger IR phenotype. In this study, levels of various PBDEs were assessed in subcutaneous and omental adipose tissues from obese IS and IR Qatari subjects and their correlation with mediators of metabolic disease were established together with their impact on insulin signaling in preadipocytes derived from these tissue.

2. Materials and Methods

2.1 Chemicals, reagents and other materials: Dichloromethane (DCM) and n-hexane were supplied by Merch (Darmstadt, Germany) or sigma Aldrich (Steinheim, Germany). All solvents were of analytical grade. Silica (200 mm) was supplied by Merch (Darmstadt, Germany). Sulfuric acid was obtained from Sigma Aldrich (Steinheim, Germany). Acidic silica gel (44%) was prepared by adding 44 g of sulfuric acid to 100 g activated silica gel, mixed well for 1 hour then stored until used. Certified standards of individual PBDE congeners (3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190, 203, 205, 206, 207, 209) (EO-5405) and $^{13}C_{12}$-labeled PBDE congeners (15, 28, 47, 99, 153, 154, 183, 197, 206, 208, 209) (EO-5426) were supplied by Cambridge Isotopoe Lab., INC. (Madover, MA, USA). Purified PBDE-47, PBDE-28, and PBDE-99 were obtained from AccuStandard Inc (New Haven, Connecticut, U.S.A). Bio-Plex Pro™ Cell Signaling Akt Panel, 8-plex (LQ00006JK0K0RR) was purchased from Bio-Rad (UK).

2.2 Cohort: Participants' recruitment criteria were described previously [5]. Briefly, 34 (22 females and 12 males) consented obese (BMI 45.4± 8.7 Kg.m^{-2}) individuals (32.1 ± 10.2 years old) undergoing bariatric surgery at AlEmadi hosptial (Doha, Qatar) were recruited. Protocols were approved by Institutional Review Board of ADLQ (X2017000224). Blood was collected prior to operation and 1–5 g abdominal SC and OM adipose tissues biopsies were collected during the surgery. Plasma cholesterol, fasting glucose and liver function enzymes were measured by COBAS INTEGRA (Roche Diagnostics, Basil). IL-6, leptin and insulin were determined using commercially available ELISA

kits. Insulin resistance was computed by homeostatic model assessment (HOMA-IR) [30] using 35th percentile (HOMAIR= 2.9) as a threshold point. Accordingly, subjects were dichotomized into IS (HOMA-IR < 2.9, n=11, 3 Males and 8 Females) and IR (HOMA-IR > 2.9, n=23, 8 Males and 15 Females).

2.3 Chemical analysis: Levels of PBDEs listed in the materials section were measured in SC and OM abdominal fat samples. Calibration solution was prepared in DCM at the following concentrations: 10-40 ng/g, 2-80 ng/g and 5-200 ng/g. Each calibration level contained $^{13}C_{12}$ labeled internal standards at 10 ng/g concentration. The labeled $^{13}C_{12}$ PBDE-congeners were spiked into the adipose tissue samples prior to extraction.

2.4 Instrumental analysis: A gas chromatography (Trace GC Ultra) coupled to a TSQ Quantum triple Quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) operated in EI positive mode at 70 eV. HP-5MS capillary column (15 m x 0.25 mm i.d. x 0.25 um film thickness) was used for the separation. The GC oven temperature was set at 120°C for 2.0 min, then at 250°C at a rate of 25°C/min, then changed to 260°C at a rate of 1.5°C/min, and finally at 300°C at a rate of 25°C/min for 15 min (the total GC run time = 31 min). Carrier gas flow rate was set at 1.2 ml/min, with constant flow mode. The triple Quadrupole was operated in multiple reaction monitoring (MRM) mode. The transfer line and ion source were kept at 310°C and 250°C, respectively. Two transitions were monitored using argon as the collision gas. The MS/MS collision energy was optimized for each PBDE

congener. Collision gas pressure was set at 1.5 m Torr. The GC was equipped with a programmable temperature vaporization (PTV) and inlet injector was set at 80°C with a split flow of 100 ml/min and a splitless time of 1.5 min. Programmed injection phase was as follows: injection time was 0.2 min; evaporation was set at 200°C with 14.5°C/sec rate for 1.0 min; transfer was set at 250°C with 14.5°C/sec and for 1.0 min and finally cleaning was set at 200°C with 14.5°C/sec rate for 1.0 min and with 50 ml/min flow (selection of evaporation and cleaning phase). All the acquisition of MS spectra was carried out in full scan mode (m/z 50 – 1000). For individual PBDE congeners, full scan spectrum was tested to identify the parent ions to be used for MS/MS analysis. The most abundant ion was selected as the parent ion. The parent ion selected for individual PBDE was isolated and the product ion scan was achieved using different collision energy values in order to identify the product ions and optimize the transition signal that provide the highest intensity. However, in addition to the selectivity obtained by MS/MS, two different transitions were monitored for each compound. For the quantification purpose, the most intense transitions were utilized. For conformation, the second transitions were used. The ratio for the two transitions were calculated for the standards and compared with the ratio found in the samples. However, as per the European Union SANCO/12495/2011 guidelines, one precursor ion with two product ions or two precursors with one product ion should be selected and applied. In the present method two precursors ion with two-product ion was adopted for all PBDE congeners. Table S1 lists parent and product ions, scan time and collision energies (eV) for individual PBDEs and 13C-PBDEs.

2.5 Pressurized liquid extraction (PLE): The Accelerated Solvent Extraction (ASE) 350 (Dionex, Sunnyvale, CA) was used for the extraction of all adipose tissue samples. 100 mg of adipose tissue were mixed with 1 g of anhydrous sodium sulfate. The ASE cell (22 ml) were filled as follows (from bottom to the top): add first the hydromatrix/ Na_2SO_4, followed by adding 10 g acidic silica (44%) and adipose tissue (100 mg), the tissue is spiked with labeled ($^{13}C_{12}$-PBDE) (10 ng) and finally the cell filled with hydromatrix / Na_2SO_4 and closed tightly. The ASE contains cellulose filter (30 mm) diameter, and 10 microns steel filter, which all are supplied by Dionex corporation (Sunnyvale, CA). The ASE cell placed in a direction, where the solvent flows from the top through the cell to the collection bottles. The extraction was achieved with H:DCM (1:9 v/v) solvent extraction. The ASE conditions were set as follows: two extraction cycles at a temperature of 100^{o}C. The static time was set at 5 min, with 66% flush volume and 120s purge time. The collection volume was 35-40 ml and finally concentrated to 100 µl using rotary and turbo evaporator, which are ready for GC-MS (MRM) injection. The lipid content is measured gravimetrically to ensure that a clean extract is obtained by ASE extraction and clean-up method. Lipids were determined gravimetrically using an aliquot of the extract and the PBDEs concentration are reported as ng/g. Fat samples (~100 mg) were weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards.

2.6 Quality assurance/quality control: The extraction, cleanup, and fractionation steps were evaluated by measurement of the absolute recoveries of the internal standards. The peaks were quantified as target compounds if: (1) the retention time matched that of the standard compound within±0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. The ratio of the m/z of the quantifying product ion and the m/z of the confirming product ion must not exceed ±15% of the mean value for the standard. The limit of detection (LOD) was calculated as three times the standard deviation of mean blank measurements and limit of quantification (LOQ) was calculated as ten times the standard deviation of mean blank measurements. If the calculated concentration in the samples was below the LOD, the concentration was not reported. Procedural blanks were analyzed simultaneously with every batch of seven samples to check for interferences or contamination from solvent and glassware. Procedural blanks were consistent (RSD, 30%) and therefore the mean value was calculated for each compounds and subtracted from the values in the samples. The recovery of the internal standards ranged from 74-139.6% and the relative standard deviations were between 0.11 to 36 %.

2.7 Preadipocytes culture and differentiation: Stromal vascular fraction (SVF) cells were obtained by collagenase digestion of adipose tissues as described previously [5]. Cell pellets were re-suspended in stromal medium containing Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM-F12) supplemented with 10% fetal bovine serum (FBS) and Penicillin/Streptomycin, then maintained at 37 °C with 5% CO_2 until confluence. To induce differentiation, early passaged

SVF-derived preadipocytes (passages 1–3) were grown at 2×10^4/cm2 in stromal medium (DMEM-F12, 10% FBS) overnight then incubated in differentiation medium (DMEM-F12, 3% FBS, 33 μM biotin, 17 μM Dpantothenate, 1 μM dexamethasone, 250 μM of methylisobutylxanthine, 0.1 μM human insulin, 5 μM of PPARγ agonist, rosiglitazone) for 7 days, followed by 12 days in maintenance medium containing same components as differentiation medium omitting methylisobutylxanthine and rosiglitazone. Differentiation potential (adipogenic capacity) was determined as a percentage of lipidtox positive stained cells to total number of stained nuclei (DAPI).

2.8 Insulin signaling analysis in response to PBDEs treatment: The effect of PBDEs treatment on the phosphorylation of AKT (Ser^{473}), BAD (Ser^{136}), GSK-3α/β (Ser^{21}/Ser^{9}), Insulin receptor substrate 1 (IRS-1) (Ser^{636}/Ser^{639}), mammalian target of rapamycin (mTOR) (Ser^{2448}), p70 S6 kinase (Thr^{389}), Phosphatase and tensin homolog (PTEN) (Ser^{380}) and S6 ribosomal protein (Ser^{235}/Ser^{236}) was quantified in human OM preadipocytes from two IS individuals. Briefly, preadipocytes were treated with 1 and 3μM of PBDE 99, 28 and 47 for 6 hours then lysed using RIPA buffer. Equal volumes of lysates were assayed using a commercial Bio-Plex Pro™ Cell Signaling Akt Panel using Luminex FLEXMAP 3D® system (Luminex Corporation) following manufacturer's instructions.

2.9 Data analysis: Data are presented as mean±standard deviation (SD) for parametric data, and mean±SEM in figures. Comparisons were performed with t-test, Wilcoxon–Mann–Whitney, 1-way ANOVA, or

Linear regression model as appropriate using IBM SPSS statistics 25. The concentrations of PBDEs were expressed in ng/g lipid weight (lw). Analyses with a p-value of 0.05 were considered significant.

3. Results

3.1 General characteristics of study population

34 obese participants were recruited from amongst patients undergoing bariatric surgery at AlEmadi Hospital (Doha, Qatar) (Table 1). Participating subjects exhibited hyperleptinemia (60.8±27.1 ng/ml) and hyperinsulinemia (15.6±8.1 mIU/L) and were dichotomized into IS and IR groups based on their HOMA-IR index. Compared to age and BMI-matched IS subjects, IR individuals had higher blood pressure, total cholesterol, ALT and AST (**Table1**).

Table 1. General characteristics of study participants.

Variables	Cohort (n = 34)	IS (N = 11)	IR (N = 23)	*p* value
Age (year)	32.1 (10.2)	32.8 (9.0)	31.8 (11.0)	0.79
BMI ($kg.m^{-2}$)	45.4 (8.7)	42.5 (8.8)	46.9 (8.5)	0.17
SBP (mmHg)	126.9 (12.5)	119.9 (9.3)	130.1 (12.7)	0.03
DBP (mmHg)	69.6 (13.5)	61.7 (15.8)	73.3 (10.8)	0.02
MAP	89.8 (10.3)	84.4 (8.7)	92.3 (10.5)	0.05
Cholesterol (mmol/L)	4.6 (0.8)	4.4 (0.8)	4.7 (0.9)	0.45
LDL (mmol/L)	2.9 (0.8)	2.7 (0.9)	2.9 (0.7)	0.55
HDLC (mmol/L)	1.1 (0.4)	1.3 (0.4)	1.2 (0.3)	0.60
Triglyceride (mmol/L)	1.3 (1.1)	1.1 (0.4)	1.4 (1.3)	0.44
Leptin (ng/ml)	60.8 (27.1)	60.1 (26.8)	61.2 (28.1)	0.93
Adiponetin (ng/ml)	3.1 (1.6)	2.5 (1.6)	3.3 (1.6)	0.40
IL-6 (pg/ml)	4.3 (2.2)	4.2 (2.3)	4.3 (2.1)	0.93
Albumin (g/L)	39.6 (3.9)	38.8 (3.5)	40.1 (4.2)	0.44
ALP (IU/L)	78.9 (22.3)	72.2 (14.4)	83.1 (25.7)	0.23
ALT (IU/L)	21.3 (10.6)	15.7 (6.2)	24.8 (11.5)	0.03
AST (IU/L)	17.5 (5.3)	14.5 (4.8)	19.4 (4.9)	0.02
Billirubin (µmol/L)	6.4 (3.6)	6.6 (2.7)	6.3 (4.1)	0.82
FBG (mmol/L)	6.6 (3.0)	5.9 (1.5)	6.9 (3.5)	0.40
Insulin (mIU/L)	15.6 (8.1)	7.9 (3.2)	19.4 (6.9)	0.00
HOMA-IR	4.2 (2.1)	2.0 (0.8)	5.3 (1.7)	0.00

BMI (body mass index), SBP (systolic blood pressure), DBP (diastolic blood pressure), MAP (mean arterial blood pressure), LDL (low density lipoprotein), HDL (high density lipoprotein), IL-6 (interleukin 6), FPG (fasting blood glucose), HOMA-IR (homeostatic model assessment of insulin resistance). Data are presented as mean (SD). Differences between (IS vs IR) were tested by the independent-sample t test or Mann–Whitney U test.

3.2 Prevalence of PBDEs in SC and OM tissues from obese individuals from Qatar

Among the tested PBDEs, only 22 were detected in SC and/or OM tissues. Using a non-targeted approach, a list of 22 identified PBDEs was created based on their molecular weights and peak heights. An orthogonal partial least squares discriminant analysis (OPLS-DA) analysis comparing PBDEs from IS and IR revealed two class-discriminatory components (Figure 1). The corresponding loading score, shown in Figure 1B, shows a number of PBDEs that were enriched in IR group (circled) including PBDE28, 47 and 99.

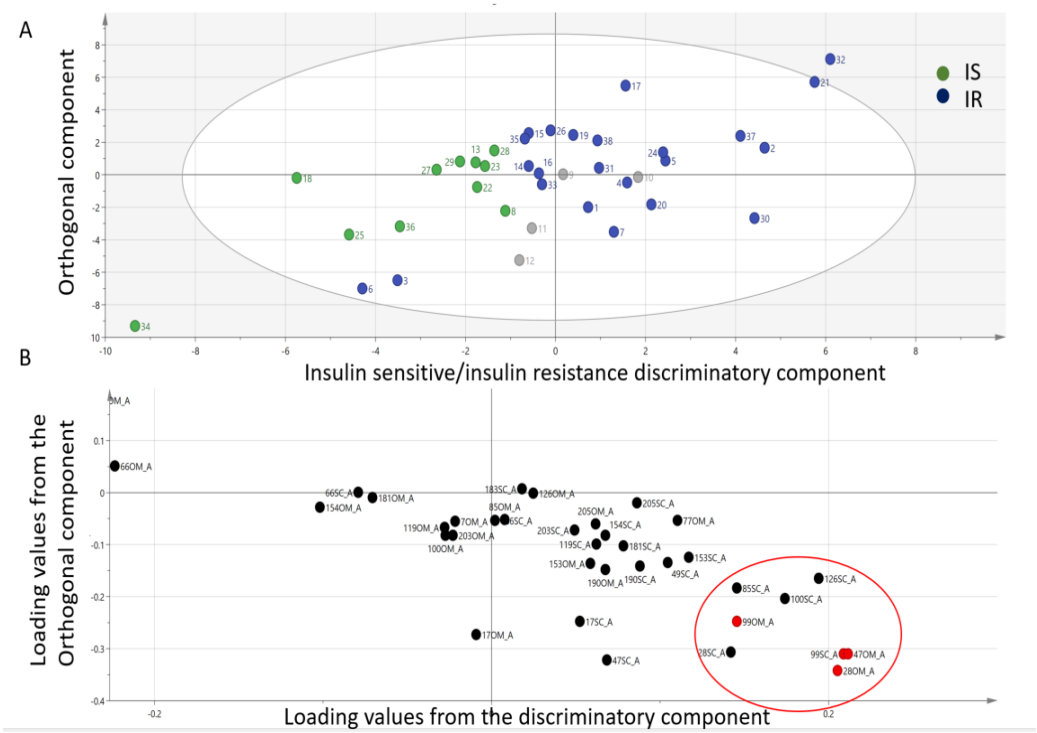

Figure 1. OPLS-DA model comparing adipose tissues-derived PBDEs from IS and IR individuals. **A)** A score plot showing the class-discriminatory component (*x*-axis) versus orthogonal component (*y*-axis). Grey circles indicate samples that were excluded because of missing data. **B)** The corresponding loading plot showing a clustering of certain PBDEs with IR is circled in red.

Levels of PBDEs 7, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 166, 181, 183, 190, 203 and 205 were detected in SC and OM adipose tissues from 34 obese participants. Table 2 summarizes prevalence of each PBDE in tested tissues and detected levels. PBDEs 28 was more prominent in both SC and OM tissues with higher accumulation in the OM tissues. Other abundant congeners included PBDE47, PBDE99 and PBDE153.

Table 2. Prevalence and levels of PBDEs congeners (ng/g tissue) in SC and OM tissues from obese Qatari individuals.

ID PBDE		LOD (ng/g)	LOQ (ng/g)	SC		OM	
				% > LOQ	Median (IQR)	% > LOQ	Median (IQR)
Mono	PBDE7	0.06	0.2	7.9	2.7 (1.42–1.42)	10.5	3.6 (0.47–171.2)
TRI	PBDE17	0.119	0.206	31.6	0.3 (0.17–0.45)	42.1	0.9 (0.21–1.63)
TETRA	PBDE28	0.281	0.936	55.3	0.4 (0.15–1.26)	65.8	1.2 (0.4–2.5)
	PBDE49	0.225	0.397	26.3	0.4 (0.2–0.8)	26.3	1.1 (0.6–1.8)
	PBDE71	0.122	0.408	0	0	2.6	2.0 (2–2)
	PBDE47	0.03	0.243	42.1	1.4 (0.7–1.6)	55.3	3.7 (1.6–17.9)
	PBDE66	0.103	0.163	21.1	0.41 (0.3–0.5)	31.6	0.6 (0.6–1.7)
	PBDE77	0.299	0.999	7.9	0.8 (0.7–0.7)	23.7	0.7 (0.6–1.9)
PENTA	PBDE100	0.119	0.273	28.9	0.4 (0.5–0.5)	34.2	0.5 (0.2–1.1)
	PBDE119	0.036	0.015	10.5	0.4 (0.3–1.9)	13.2	0.5 (0.2–1.5)
	PBDE99	0.397	0.623	39.5	0.5 (0.3–0.7)	44.7	1.0 (0.4–3.7)
	PBDE85	0.088	0.109	18.4	0.3 (0.1–0.5)	13.2	0.7 (0.3–1.5)
	PBDE126	0.035	0.118	18.4	0.4 (0.2–2.2)	10.5	0.9 (0.3–8.6)
HEXA	PBDE154	0.059	0.161	13.2	1.8 (1.2–3.4)	15.8	0.8 (0.2–1.7)
	PBDE153	0.009	0.026	36.4	0.6 (0.5–1.2)	23.7	0.6 (0.5–1.7)
	PBDE138	0.008	0.038	7.9	1.2 (0.5–0.5)	5.3	2.5 (1.7–1.7)
	PBDE166	0.012	0.039	10.5	2.2 (1–3.3)	2.6	1.4 (1.4–1.4)
HEPTA	PBDE183	0.039	0.105	15.8	2.4 (1.2–3.7)	7.9	4.3 (1.3–1.3)
	PBDE181	0.078	0.194	13.2	4.9 (2.0–6.4)	13.2	3.6 (1.6–11.6)
	PBDE190	0.206	0.686	13.2	2.2 (1.3–8.1)	21.1	3.7 (0.6–10.5)
OCTA	PBDE203	0.053	0.178	13.2	3.1 (0.3–10.7)	28.9	5.7 (0.6–15.1)
	PBDE205	0.102	0.121	18.4	3.5 (2.5–8.4)	21.1	4.0 (1.0–9.6)

Data are presented as median (IQR).

3.3 Correlation of PBDEs with mediators of metabolic syndrome

Correlations between levels of detected PBDEs and mediators of metabolic syndrome were identified (Table 3A). There were significant negative correlations between PBDE47 in the SC tissues and circulating HDL cholesterol and IL-6 levels and between levels of PBD203 in the OM tissue and triglyceride levels. Significant positive correlations were found between levels of PBDE99 in SC tissue with Leptin and IL-6 and between PBDE47 in the OM tissue with ALP and insulin levels. Significant positive correlations were similarly identified between insulin and PBDEs 47 and 99 in the OM tissues. When considering correlations between levels of PBDEs themselves (Table 3B), there were significant correlations between PBDE28 in the SC and OM tissues and between levels of PBDE 28 in the OM tissues and PBDEs 17, 47 and 99 in the same tissues. Similarly, there were significant positive correlations between PBDE47 in the OM tissue and levels of PBDEs 28 and 99 in the same tissue. There were no significant correlations between any of the detected PBDEs and the adipogenic capacity of SC or OM tissue-derived preadipocytes (data not shown).

Table 3. Spearman correlation coefficients between detected PBDEs and mediators of metabolic syndrome (A) and other PBDEs (B).

A	HDLC	TG	Leptin	IL6	ALP	Insulin
SC-PBDE47	−0.573*	−0.446	0.122	−0.733*	−0.003	−0.156
SC-PBDE99	−0.014	0.228	0.786*	0.738*	0.378	0.352
OM-PBDE28	−0.258	0.096	−0.124	−0.093	0.283	0.396
OM-PBDE47	−0.239	−0.383	0.029	−0.118	0.676*	0.530*
OM-PBDE99	−0.104	0.327	−0.545	−0.218	0.496	0.536*
OM-PBDE203	0.357	−0.881**	0.750	−0.250	−0.430	0.227

B	SC-PBDE28	SC-PBDE47	SC-PBDE99	OM-PBDE17	OM-PBDE28	OM-PBDE47	OM-PBDE99
SC-PBDE28	1.000	0.755**	0.441	0.100	0.629*	0.524	0.543
SC-PBDE47	0.755**	1.000	0.267	0.400	0.524	0.800	0.200
OM-PBDE28	0.629*	0.524	0.571	0.657*	1.000	0.561*	0.621*
OM-PBDE47	0.524	0.800	0.200	0.400	0.561*	1.000	0.698**
OM-PBDE99	0.543	0.200	0.500	0.559	0.621*	0.698**	1.000

(* p< 0.05, ** p<0.01).

5

3.4 Insulin-resistance associated PBDEs

Levels of detected PBDEs were compared between IS and IR subjects. PBDE99 levels within the SC tissues were 2.3 fold higher in IR-derived PBDE (p=0.05) than IS counterparts (Figure 2). Similarly, two different PBDEs isolated from OM tissues were significantly higher in tissues derived from IR subjects compared to IS counterparts (Figure 2). These include PBDE28 and PBDE47 with 1.7 (p=0.02) and 5.3 (p=0.02) fold difference respectively. A similar trend was seen in PBDE99 in the OM tissue with 2.8 fold increase in IR compared to IS tissues (p=0.09) (Figure 2). When considering PBDEs congeners, penta congeners including PBDE100, 119, 99, 85, and 126 were also higher in IR individuals compared to IS counterparts by 2.1 fold (p=0.01), while there were no significant differences in mono, tri, tertra, hexa, hepta and octa congeners between the two studied groups.

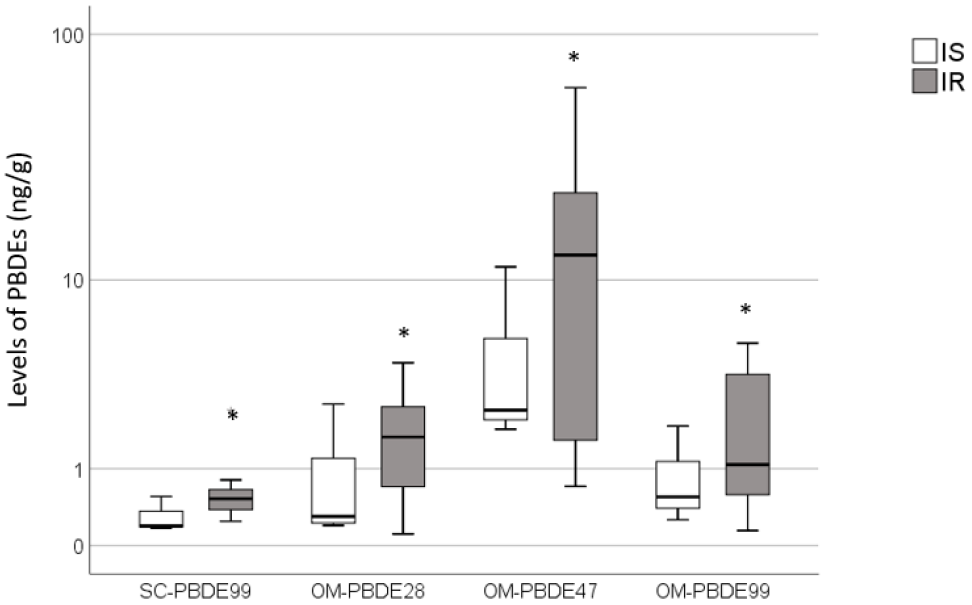

Figure2. Levels of PBDEs in subcutaneous (PBDE99) and omental (PBDE28, 47 and 99) tissues in relation to insulin resistance. Data are presented as Median (IQR). * P<0.05 by independent samples T-test using log transformed data.

3.5 Analysis of insulin signaling in IS OM preadipocytes in response to PBDEs treatment

Significant differences in the phosphorylation of mediators of insulin signaling pathway were detected in OM preadipocytes treated with 1 and 3μM of PBDE 28, 47 and 99 (Figure 3A-C). PBDE28 caused inhibition of phosphorylation of GSK3 α/β (Ser^{21}/Ser^{9}), mTOR (Ser^{2448}), p70 S6 kinase (Thr^{389}), and S6 ribosomal protein (Ser^{235}/Ser^{236}) and activation of PTEN (Ser^{380}) phosphorylation (Figure 3A), suggesting inhibition of insulin signaling. Interestingly, IS-derived preadipocytes exhibited higher levels of phosphorylated BAD (Ser^{136}) in response to all PBDEs treatments, suggesting a pro-apoptotic effect of PBDE treatment.

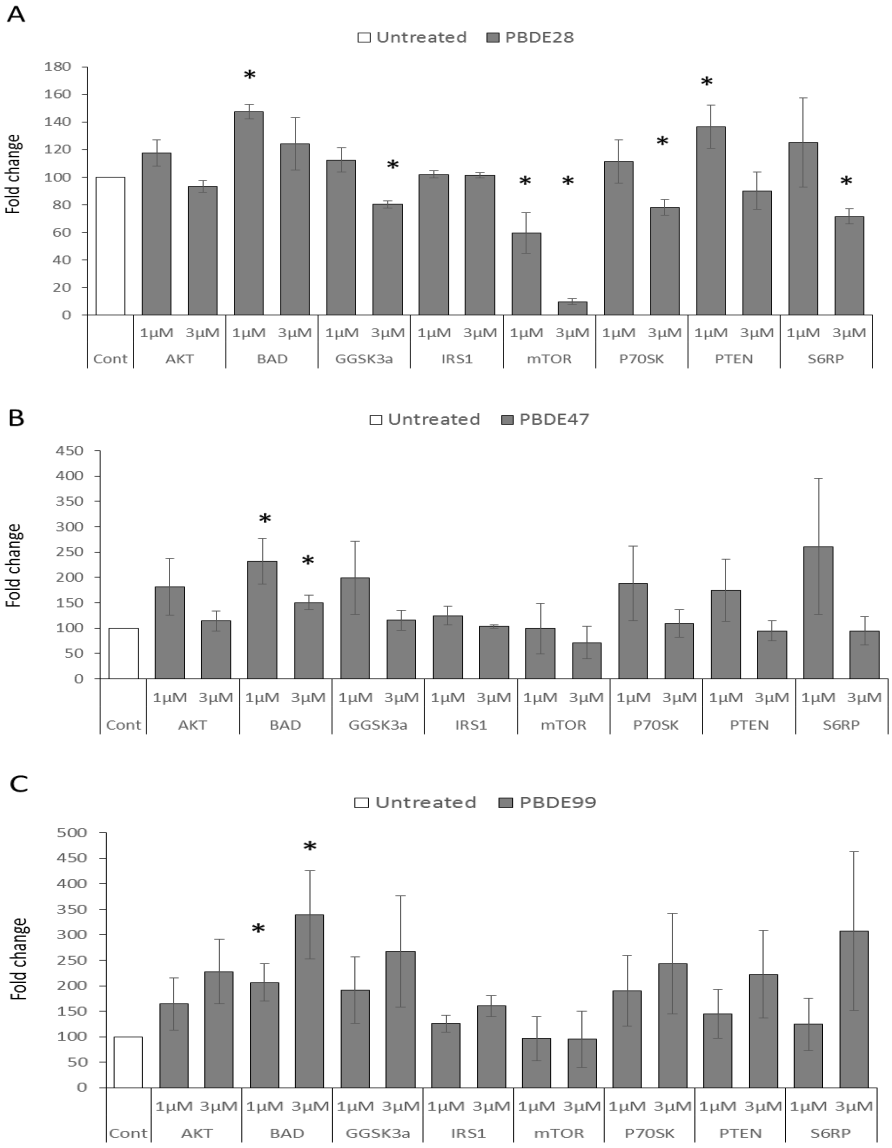

Figure 3. Effect of PBDEs on insulin signaling in OM preadipocytes derived from two insulin sensitive (IS) individuals. Levels of phosphorylated AKT (Ser473), BAD (Ser136), GSK-3α/β (Ser21/Ser9), IRS-1 (Ser636/Ser639), mTOR (Ser2448), p70 S6 kinase (Thr389), PTEN (Ser380), p70 S6 kinase (Thr389) and S6 ribosomal protein (Ser235/Ser236) were measured following 6 hours treatment of omental preadipocytes from IS subjects to 1-3μM of PBDE28, 47 and 99. Data are presented as Mean±SEM. * $P<0.05$ by ANOVA followed by independent samples T-test. N=4 per treatment.

4. Discussion

This pilot study is the first to compare the bioaccumulation of PBDEs in adipose tissues from obese Qatari IS and IR individuals.

Levels of various PBDEs belonging to different congeners were assessed in SC and OM adipose tissues from 34 obese (11 IS and 23 IR) and their correlation to mediators of metabolic disease including age, BMI, lipid profiles, inflammatory markers and liver function enzymes were investigated. Our results suggested that out of the 22 detectable PBDEs in SC and OM adipose tissues, the predominant PBDE species (PBDE99, 28 and 47) were significantly higher in IR individuals compared to the IS counterparts. Although none of the penta-BDEs was associated individually with insulin resistance, they collectively were significantly different between the two groups. On the other hand, PBDEs 28 and 47 were individually associated with insulin resistance but when combined with other members of their respective groups, were not significantly different. This may suggest that both 28 and 47 are good predictors, with a potential causal role, of insulin resistance whereas other PBDEs will only predict increased risk of insulin resistance when considered collectively (per class).

Positive correlations among PBDEs 28, 47 and 99 in the OM adipose tissues were detected our study. Although the number of participants was relatively low in light of the prevalence of identified PBDEs, similar correlations were previously reported in serum samples from 1367 participants [19], suggesting that these correlations can also be detected at the level of the visceral adipose tissues. Our data also confirm previous studies suggesting association of PBDEs within adipocytes with increased risk of metabolic disease including obesity, insulin resistance

and T2DM [23]. The association of PBDE47 with increased fasting blood insulin and insulin resistance in our cohort may be related to its role in disturbing glucose metabolism shown previously in postnatal mice exposed to PBDE47 [26]. Penta-BDEs were also associated with insulin resistance in our cohort, perhaps through modulation of the activity of thyroid hormones that share structural similarities with PBDEs [28, 31]. Disturbance in thyroid hormones balance can influence glucose metabolism, leading to the development of insulin resistance [32]. Previous studies have shown that developmental exposure to penta-BDE reduced circulating free thyroxine and T3 significantly [33-35], increased lipolysis and reduced glucose oxidation [23], although the underlying mechanisms remain unknown. The similarity between structures of PBDE metabolites and T4 and tri-iodothyronine may suggest a competition between PBDE metabolites and the thyroid hormones for binding thyroid hormone transporter transthyretin [31]. This could activate clearance of these hormones, which may have contributed to penta-BDE-induced reduction in thyroid hormone level.

We also show that PBDE28 to be associated with in our cohort, perhaps due to its positive correlation with inflammatory marker IL-6 shown previously to induce insulin resistance in adipocytes [5, 36]. The correlation between PBDEs and inflammation could provide a mechanism for predisposition of obese individuals to insulin resistance, but this suggestion needs further investigation. The association between PBDE99 and insulin resistance may be due to its correlation with PBDEs 28 and 47, although a functional role of PBDE99 remains to be investigated. Previous studies have suggested that adipogenesis of mouse preadipoctyes increase in the presence of PBDEs [29], however

our data showed no association between detected levels of PBDEs and adipogenic capacity of preadipocytes derived from SC or OM adipose tissues.

In order to investigate the effect of PBDEs on induction of insulin resistance, primary human preadipocytes from OM tissues of IS subjects were treated with PBDEs 28, 47 and 99. All PBDEs caused increased phosphorylation of BAD (Ser^{136}). The pro-apoptotic function of BAD is regulated by phosphorylation of two sites, serine-112 and serine-136 (Ser-136), resulting in loss of the ability of BAD to form a heterodimer with the survival proteins BCL-XL or BCL-2 [37]. Thus, treatment of preadipocytes with PBDEs seems to have triggered a pro-apoptotic pathway that requires further validation. PBDE28 in particular caused inhibition of phosphorylation of GSK3 α/β (Ser^{21}/Ser^{9}), mTOR (Ser^{2448}), p70 S6 kinase (Thr^{389}) and S6 ribosomal protein (Ser^{235}/Ser^{236}), suggesting inhibition of insulin signaling. PBDE28 induced of the phosphorylation of PTEN (Ser^{380}) that negatively regulates insulin/PI3K signaling [38], confirming its potential role as inhibitor of insulin signaling and activator of insulin resistant phenotype. A similar trend was seen with PBDE47 but did not reach statistical significance. Recent work showed that perinatal exposure to environmentally relevant doses of PBDE47 in mice caused activation of mTOR complexes and prolonged changes in liver metabolic programming. This evidence further confirms the ability of PBDEs to affect the mTOR pathway, the pathway involved in the pathogenesis of conditions representing major public health threat [39].

5. Conclusion

In summary, this study identifies for the first time PBDEs levels in adipose tissues from the Arabian Peninsula region. Emerging data shows that bioaccumulation of specific PBDEs in human adipose tissues is associated with insulin resistance in obese individuals and can trigger insulin resistant phenotype *in vitro*. While it is premature to extrapolate these findings on the functional roles of these PBDEs *in vivo* and their potential associations with diabetes epidemic seen in this region, our findings support the need for further investigation of their potential endocrine-disrupting effects on human adipocyte functionality. Because of their bioaccumulation and persistence in the environment, PBDEs will continue to constitute risk to humans and animals for many years to come. Hence it is critical that we understand the potential health effects associated with this exposure.

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Competing interests

None of the authors have any competing interests in the manuscript.

Author's contributions

MH and NT conducted PBDE analytical analysis in adipose tissues, ID conducted the statistical and bioinformatics analyses, NM, HS and AM performed insulin signaling experiments. ME consented participants and collected samples. AL and MAE collected data, carried out analysis and wrote the paper. All authors reviewed and accepted the paper's final version. MAE (corresponding) is responsible for the integrity of the work as a whole.

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Chapter 6: Summary and Future Prospects

Summary and Future Prospects:

Obesity is associated with increased risk of metabolic syndrome including cardiovascular disease, hypertension, cancer and type 2 diabetes mellitus (T2DM). However, not all obese individuals fall victims for obesity-associated comorbidities as a subset of obese subjects, known as the obese insulin sensitive obese (OIS), show lower comorbidities compared to their BMI-matched obese insulin resistant (OIR) counterparts. Those with lower risk maintain insulin sensitivity and show lower systemic lipids as well as inflammatory and oxidative stress markers. Various factors were suggested to influence inflammatory and oxidative stress mediators associated with the higher risk group including genetic and environmental factors. While evidence for genetic predisposition is still to be substantiated, the environmental factors including specific pollutants and medications were previously suggested to play a role. In order to investigate the molecular pathways associated with the protective mechanisms in OIS, this thesis describes an OMICS approach that studied the potential underlying pathways that differentiate the OIS and OIR in blood and adipose tissues.

Our lab has previously highlighted the role of impaired adipogenesis in increased risk of insulin resistance in obese individuals. **In chapter 2** of this thesis, we provide a full review summarizing recent literature of mediators of impaired adipogenesis associated with insulin resistance and its underlying molecular pathways including obesity-associated mild chronic inflammation, oxidative stress, fatty acid signaling and environmental factors.

In order to investigate the metabolic profiles associated with increased risk of insulin resistance in OIR and T2DM compared to OIS, **chapter 3** describes our data using untargeted metabolomics analysis of blood samples from lean controls, OIS, OIR and obese-T2DM individuals. Our data reveal novel phospholipid metabolites (choline, glycerophosphoethanolamine and glycerophosphorylcholine) that were significantly altered in OIS when compared with OIR and T2DM individuals. In addition, we confirmed metabolites that were previously shown to be associated with insulin resistance and T2DM (such as glucose and 1,5-anhydroglucitol) and metabolites that were significantly associated with obesity-related comorbidities (such as 3-hydroxylaurate, 3-hydroxymyristate, homoarginine and dimethylarginine). We discussed the potential roles of these metabolites and highlighted the importance of further studies to confirm these associations in other cohorts and investigate their functionality.

Following metabolic profiling of blood samples, we sought lipidomics analysis of adipose tissues in order to identify triacylglycerol-specific lipidomic signatures associated with insulin resistance and T2DM. **chapter 4** describes our data from these studies used to investigate differences between subcutaneous (SC) and mental (OM) adipose tissues that constitute the two main fat depots with direct roles in the etiology of insulin resistance. Although triacylglycerols (TAGs) themselves are unlikely to be signaling molecules, fatty acids produced during their synthesis or breakdown were shown to interfere with the intracellular insulin signaling pathways leading to development of insulin

resistance. Our finding identified 76 unique TAG species in adipose tissues classified based on their exact mass. Furthermore, our data revealed a number of TAG levels that were significantly altered with disease progression including C46:4, C48:5, C48:4, C38:1, C50:3, C40:2, C56:3, C56:4, C56:7 and C58:7. When looking into their fatty acid composition, two fatty acids were identified by enrichment analysis including C12:0 fatty acid that was increased in OIS and C18:3 that was enriched in both depleted and enriched TAGs in T2DM. We observed associations of identified TAGs species with metabolic traits, including age and body mass index, systemic total cholesterol, TAGs, and interleukin-6 in addition to adipogenic potential of preadipocytes derived from the same adipose tissues. These associations could shed light on the molecular mechanisms contributing to the increased risk of metabolic disease. Future experiments are needed to investigate the functional significance of these specific lipidomic profiles.

Once metabolites differentiating OIS and OIR were identified, we sought to investigate the environmental factors that could also participate in the increased risk of insulin resistance in the OIR group. Polybrominated diphenyl ethers (PBDEs) are brominated synthetic compounds that have been used as flame retardants. PBDEs accumulate in adipose tissue causing disruption of its endocrine function. Exposure to PBDEs has been found to increase risk of insulin resistance. **Chapter 5** describes an analysis of the bioaccumulation of specific classes of PBDEs in SC and OM tissues from OIS and the OIR. Our method involved assessment of levels of different PBDE congeners in SC and OM adipose tissues from obese Qatari individuals using gas

chromatography (Trace GC Ultra) coupled to a TSQ Quantum triple Quadrupole mass spectrometer and their correlation with mediators of metabolic disease. Our data showed 22 detectable PBDEs in SC and OM tissues including PBDEs 28, 47, 99 and 153 that were predominant in omental adipose tissues from obese Qatari subjects. Our data also indicated that PBDEs 99, 28, and 47 that were significantly higher in OIR individuals compared to their OIS counterparts. We have also identified significant positive correlations between PBDEs 28 and 99 in the OM tissues and fasting insulin levels. When considering PBDEs congeners, penta congeners were also higher in OIR compared to OIS individuals, while no significant differences were detected in mono, tri, tertra, hexa, hepta and octa congeners between the two studied groups. Treatment of human omental preadipocytes from insulin sensitive individuals with PBDE28 caused inhibition of phosphorylation of GSK3 α/β (Ser^{21}/Ser^{9}), mTOR (Ser^{2448}), p70 S6 kinase (Thr^{389}) and S6 ribosomal protein (Ser^{235}/Ser^{236}) and activation of PTEN (Ser^{380}) phosphorylation, suggesting inhibition of insulin signaling. Findings in this chapter suggest a direct role of PBDEs in the increased risk of obesity-associated insulin resistance and identify their species that are most associated with increased risk.

Collectively, our data identify metabolites differentiating OIS and OIR and characterize the role of specific environmental pollutants on increased risk of obesity-associated insulin resistance. Future work will include confirmation of these associations in different cohorts as well as their functional validation by studying their role in insulin signaling in the presence and absence of various inflammatory and oxidative

stress mediators and environmental pollutants. Future work will also investigate the role of these metabolites in increasing risk of insulin resistance in lean subjects, aiming ultimately for the assessment of their utilization as potential diagnostic biomarkers and/or therapeutic targets.

Chapter 7: Samenvatting en verder onderzoek

Samenvatting en verder onderzoek

Obesitas gaat gepaard met een verhoogd risico op stofwisselingsproblemen zoals optreden bij hart- en vaatziekten, hoge bloeddruk, kanker en diabetes type 2 (DM2). Niet iedereen met obesitas krijgt echter obesitasgerelateerde aandoeningen. Het blijkt dat een groep obese individuen die bekend staan als obese insulinesensitieven (OIS) minder comorbide aandoeningen hebben dan obese insulineresistenten (OIR) die met hen gematcht zijn op basis van BMI. Degenen met het lagere risico blijven gevoelig voor insuline en hebben lagere systemische lipidewaarden en minder inflammatoire en oxidatieve stressmarkers. Verondersteld werd dat diverse factoren (bijvoorbeeld genetische en omgevingsgebonden factoren) de inflammatoire en oxidatieve stressmediatoren in de hogere risicogroep beïnvloeden. Hoewel er nog geen bewijs is dat genetische aanleg een rol speelt, werd vroeger aangenomen dat omgevingsfactoren zoals specifieke verontreinigingen en medicijnen wel van invloed zijn. Dit proefschrift beschrijft een OMICS-aanpak waarbij de mogelijke onderliggende routes onderzocht worden die in bloed en vetweefsel het onderscheid maken tussen OIS en OIR, om zicht te krijgen op de moleculaire routes die een rol spelen bij OIS beschermingsmechanismen.

Onderzoekers van ons laboratorium hebben eerder de rol beschreven die een verstoorde vorming van vetcellen (adipogenese) speelt bij een verhoogd risico op insulineresistentie in obese individuen. **Hoofdstuk 2** van dit proefschrift beschrijft een onderzoek van alle recente

literatuur over mediatoren van verstoorde adipogenese die verband houden met insulineresistentie en de onderliggende moleculaire routes, zoals obesitasgerelateerde lichte chronische ontsteking, oxidatieve stress, vetzuursignalering en omgevingsfactoren.

Om de metabole profielen die verband houden met een verhoogd risico op insulineresistentie bij OIR en DM2 te vergelijken met OIS, beschrijft **hoofdstuk 3** de uitkomsten van non-specifieke metabolische analyses van bloedmonsters genomen van OIS, OIR en obese DM2 patiënten en een controlegroep met een normaal gewicht. De data laten nieuwe fosfolipidemetabolieten (choline, glycerofosfoethanolamine en glycerofosforylcholine) zien die significant anders zijn in OIS individuen dan in OIR en DM2 individuen. Bovendien vonden we metabolieten waarvan al eerder is aangetoond dat ze verband houden met insulineresistentie en DM2 (zoals glucose en 1,5-anhydroglucitol) en metabolieten die significant verband houden met obesitasgerelateerde comorbiditeit (zoals 3-hydroxylauraat, 3-hydroxymyristaat, homoarginine en dimethylarginine). We gaan in op de mogelijke rol die deze metabolieten spelen en benadrukken het belang van verder onderzoek om deze verbanden te bevestigen in andere cohorten en meer te weten te komen over hun functies.

Na metabolische profilering van de bloedmonsters voerden we een lipidomische analyse uit van vetweefsel om triacylglycerol-specifieke lipidesignaturen te identificeren die verband houden met insulineresistentie en DM2. **Hoofdstuk 4** beschrijft de data uit ons onderzoek die gebruikt zijn om verschillen te onderzoeken tussen onderhuids (SC) en

omentaal (OM) vetweefsel, de twee belangrijkste opslagplaatsen van vet die een directe rol spelen bij het ontstaan van insulineresistentie. Hoewel triacylglycerolen (TAG's) zelf waarschijnlijk geen signaalmoleculen zijn, is gebleken dat vetzuren die geproduceerd worden tijdens de synthese of afbraak van TAG's de intracellulaire insulinesignaalroutes verstoren en zo leiden tot insulineresistentie. Op basis van de exacte molecuulmassa konden we 76 unieke TAG-typen in vetweefsel identificeren. Verder liet onze data zien dat een aantal TAG-concentraties – bijvoorbeeld van C46:4, C48:5, C48:4, C38:1, C50:3, C40:2, C56:3, C56:4, C56:7 en C58:7 – significant veranderen naarmate de ziekte voortschrijdt. Verrijkingsanalyse van de vetzuursamenstelling liet zien dat het C12:0 vetzuur verhoogd was bij OIS en dat C18:3 verrijkt was in zowel verarmde als verrijkte TAG's bij DM2. We zagen verbanden tussen de gevonden TAG-typen en metabolische kenmerken zoals leeftijd en BMI, systemisch totaalcholesterol, TAG's en interleukine-6, en een adipogeen potentieel van pre-adipocyten verkregen uit hetzelfde vetweefsel. Deze verbanden kunnen licht werpen op de moleculaire mechanismen die bijdragen aan een verhoogd risico op stofwisselingsziekten. Meer onderzoek is nodig om het functionele belang van deze specifieke lipodeprofielen te begrijpen.

Na identificatie van de metabolieten die onderscheidend zijn voor OIS en OIR, onderzochten we de omgevingsfactoren die ook mee kunnen spelen bij een verhoogd risico op insulineresistentie in de OIR-groep. Polybroomdifenylethers (PBDE's) zijn gebromeerde synthetische stoffen die gebruikt zijn als vlamvertragers. PBDE's hopen zich op in vetweefsel en verstoren de endocriene functie ervan. Blootstelling aan

PBDE's verhoogt het risico op insulineresistentie. **Hoofdstuk 5** beschrijft een analyse van de bioaccumulatie van specifieke PBDE-klassen in SC en OM weefsels van OIS en OIR individuen. Hiertoe bepaalden we de concentraties van diverse PBDE-typen in SC en OM vetweefsel van obese Qatarese individuen met behulp van gaschromatografie (Trace GC Ultra) gekoppeld aan een TSQ Quantum triple quadrupole massaspectrometer en hun correlatie met mediatoren van stofwisselingsziekten. De uitkomsten laten 22 detecteerbare PBDE's (waaronder PBDE's 28, 47, 99 en 153) zien in SC en OM weefsel, die het meest aanwezig waren in het omentale vetweefsel van de obese Qatarezen. De uitkomsten laten ook zien dat PBDE's 28, 47 en 99 significant hoger waren in OIR individuen dan in hun OIS tegenhangers. We zagen ook significant positieve correlaties tussen PBDE's 28 en 99 in het OM weefsel en de nuchtere insulinespiegel. Voor wat betreft het type PBDE, waren de concentraties van pentatypen ook hoger in OIR dan in OIS individuen. Voor mono-, tri-, tetra-, hexa-, hepta- en octatypen werden geen significante verschillen gevonden tussen de twee groepen proefpersonen. Behandeling van humane omentale pre-adipocyten uit insulinesensitieve individuen met PBDE28 leidde tot remming van de fosforylering van GSK3 α/β (Ser^{21}/Ser^{9}), mTOR (Ser^{2448}), p70 S6 kinase (Thr^{389}) en S6 ribosomaal eiwit (Ser^{235}/Ser^{236}) en activatie van PTEN (Ser^{380}) fosforylering, wat wijst op mogelijke remming van de insulinesignaalbaan. De bevindingen in dit hoofdstuk wijzen op een directe rol van PBDE's bij een verhoogd risico op obesitasgerelateerde insulineresistentie en laten zien welke typen het sterkst verband houden met dit verhoogde risico.

Het totaal van onze data laat zien welke metabolieten differentiëren tussen OIS en OIR en de rol die specifieke verontreinigende stoffen uit de omgeving spelen bij een verhoogd risico op obesitasgerelateerde insulineresistentie. Verder onderzoek moet deze verbanden bevestigen in andere cohorten en deze functioneel valideren door hun rol in de insulinesignaalbaan na te gaan bij aan- en afwezigheid van diverse inflammatoire en oxidatieve stressmediatoren en verontreinigende stoffen uit de omgeving. Ook moet de rol die deze metabolieten spelen bij eventuele vergroting van het risico op insulineresistentie in personen met normaal gewicht onderzocht worden, om uiteindelijk na te gaan of ze bruikbaar kunnen zijn als diagnostische biomarkers en/of aangrijpingspunten voor behandeling.

Chapter 8: الملخص والرؤية المستقبلية

الملخص والرؤية المستقبلية

ترتبط السمنة بزيادة خطر الإصابة بمتلازمة الأيض بما في ذلك أمراض القلب والأوعية الدموية وارتفاع ضغط الدم والسرطان والسكري من النوع الثاني (T2DM). وعلى الرغم من ذلك لا يعاني جميع الأفراد البدينين من توابع السمنة هذه بل إن بعضهم ويعرفون بالبدناء ذوي الحساسية للأنسلوين (OIS) يظهرون أعراضاً أقل حدة من نظرائهم المطابقين لهم في مؤشر كتلة الجسم (BMI) والمعروفين بالبدناء المقاومين للأنسلوين (OIR)، بما في ذلك نسبة دهون ومؤشرات التهابية وعوامل أكسدة منخفضة. أما عن أسباب هذه الاختلافات فإنها تتضمن عوامل وراثية وأخرى بيئية. وقد سبق أن تم اقتراح ان العوامل البيئية بما في ذلك بعض الملوثات البيئية والأدوية تلعب دوراً في زيادة خطر أو حماية هؤلاء الأفراد من توابع البدانة ، في حين أن الأدلة على العوامل الوراثية ما زالت غير مفهومة تماماً. بناءً على هذه المعطيات فقد هدفت دراستنا إلى التحقق من الآليات الجزيئية المرتبطة بحالة OIS باستخدام تحاليل OMICS والتي درست المسارات الكامنة الخاصة بالبدناء المقاومين للأنسلوين من تلك المرتبطة بالبدناء ذوي الحساسية للأنسلوين في الدم والأنسجة الدهنية.

لقد أثبتت الدراسات السابقة في مختبرنا الدور الذي يلعبه الخلل في تمايز الخلايا الجذعية الدهنية (adipogenesis)في زيادة خطر مقاومة الأنسولين لدى الأفراد الذين يعانون من السمنة المفرطة. في **الفصل الثاني** من هذه الرسالة تم تقديم مراجعة كاملة لدراسات العوامل المرتبطة والمساهمة في خلل adipogenesis في البدناء المقاومين للأنسلوين ومساراتها الجزيئية الأساسية بما في ذلك الالتهاب المزمن الخفيف المرتبط بالسمنة والإجهاد التأكسدي ودور الأحماض الدهنية والعوامل البيئية.

من أجل التحقق من العوامل الأيضية المرتبطة بزيادة خطر مقاومة الأنسولين، يصف **الفصل الثالث** نتائج استخدام تحليل untargeted metabolomics لعينات الدم من OIS و OIR و T2DM و مقارنتها بتحاليل عينات دم من أشخاص غير بدينين. كشفت نتائجنا عن تغيرات في عدة مستقلبات أيضية بما فيها (choline, phospholipid metabolites glycerophosphoethanolamine and glycerophosphorylcholine) والتي تم تغييرها بشكل كبير في OIS عند مقارنتها بأفراد OIR و T2DM. بالإضافة إلى ذلك ، أكدت

تحاليلنا نتائج دراسات سابقة أظهرت أن عدة مستقلبات أيضية ترتبط بمقاومة الأنسولين و T2DM (مثل glucose and 1,5- anhydroglucitol) و أخرى التي ترتبط بشكل كبير مع الأمراض المرتبطة بالسمنة (3-hydroxylaurate, 3hydroxymyristate, homoarginine، dimethylarginine). ناقشنا الأدوار المحتملة لهذه المستقلبات الأيضية وأبرزنا أهمية إجراء مزيد من الدراسات لتأكيد دورها والتحقق من وظائفها في مجموعه مشاركين أخرى.

بعد دراسة المستقلبات الأيضية لعينات الدم، استخدمنا تحاليل Lipidomics للأنسجة الدهنية لتحديد أنواع الدهون الثلاثية المرتبطة بمقاومة الأنسولين وT2DM. يصف **الفصل الرابع** نتائج هذه الدراسات والتي تناولت الاختلافات بين الأنسجة الدهنية تحت الجلد (SC) والأنسجة الدهنية المحيطة بالأعضاء (OM) اللتان تشكلان مستودعي الدهون الرئيسيين ذوي الدور مباشر في عملية مقاومة الأنسولين. وعلى الرغم من أن الدهون الثلاثية نفسها ليست فاعلة إلا أن الاحماض الدهنية التي تم انتاجها أثناء تصنيع أو تكسير الدهون هي التي تتداخل مع مسارات إشارات الأنسولين داخل الخلايا مؤدية إلى تطور مقاومة الأنسولين. أظهرت نتائجنا وجود 76 نوعا من الشحوم الثلاثية في الأنسجة الدهنية والتي تم تصنيفها بناء على كتلتها الجزيئية. علاوة على ذلك، كشفت بياناتنا عن عدد من الشحوم الثلاثية التي تم تغييرها بشكل كبير مع تطور المرض بما في ذلك C46: 4، C48: 5، C48: 4، C38: 1، C50: 3، C40: 2، C56: 3، C56: 4، C56: 7 و C58: 7. عند النظر في تكوين الأحماض الدهنية الخاصة بتلك الشحوم الثلاثية، تم تحديد اثنين من الأحماض الدهنية التي تغيرت مع المرض بما في ذلك الحامض الدهني C12: 0 الذي تم زيادته في OIS و C18: 3 والذي كان تغير مع وجود T2DM. كما لاحظنا وجود ارتباطات بين أنواع شحوم ثلاثية وسمات أيضية معينة بما في ذلك مؤشر كتلة العمر والجسم ، والكوليسترول ومستوى الشحوم الثلاثية في الدم و 6-interleukin بالإضافة إلى إمكانية القدرة التمايزية للخلايا الجذعية الدهنية المستمدة من الأنسجة الدهنية نفسها. ساعد تحديد هذه العلاقات على فهم الآليات الجزيئية التي تساهم في زيادة خطر الإصابة بالأمراض الأيضية مع التأكيد على أهمية التحقق من هذه النتائج في دراسات مستقبلية أخرى.

بعد ان تم تحديد المستقلبات الأيضية المميزة في OIS و OIR، تحققنا من العوامل البيئية التي يمكن أن تشارك أيضا في زيادة خطر مقاومة الأنسولين في مجموعة OIR. وتعد Polybrominated

diphenyl ethers (PBDEs) وهي مركبات اصطناعية تستخدم كمثبطات للحريق إحدى تلك العوامل. تتراكم PBDEs في الأنسجة الدهنية مسببة اختلال وظائف الغدد الصماء. وقد تبين أن التعرض الى PBDEs يزيد من خطر مقاومة الأنسولين. يصف **الفصل الخامس** تحليلًا لتراكم فئات معينة من PBDEs في أنسجة SC و OM من OIS و OIR. تضمن بحثنا تقييماً لأنواع PBDEs في الأنسجة الدهنية المستخلصة من SC و OM من الأفراد القطريين الذين يعانون من السمنة المفرطة باستخدام gas chromatography (Trace GC Ultra) coupled to a TSQ Quantum triple Quadrupole mass spectrometer TSQ. أظهرت بياناتنا 22 نوع من PBDEs في أنسجة SC و OM بما في ذلك 28 و 47 و 99 و PBDEs153 التي كانت أكثر شيوعاً في الأنسجة الدهنية OM من الأشخاص القطريين الذين يعانون من السمنة المفرطة. أشارت بياناتنا أيضًا إلى أن 99 و 28 و PBDEs47 كانت أعلى بكثير في الأفراد الذين يعانون من OIR مقارنة بنظرائهم في OIS. لقد حددنا أيضًا علاقات بين نسب PBDEs 28 و 99 في أنسجة OM ومستويات الأنسولين في الدم. عند النظر في متجانسات PBDEs ، كانت المتجانسات الخماسية أعلى أيضًا في OIR مقارنة بأفراد OIS ، في حين لم يتم اكتشاف فروق ذات دلالة إحصائية في المتجانسات الأخرى بين المجموعتين اللتين تمت دراستهم. كما بينت نتائجنا أن المعالجة المسبقة للخلايا الجذعية الدهنية المستخلصة من البدناء ذوي الحساسية للأنسلوين ب PBDE28 تسببت في تثبيط الحساسية للأنسولين وبذلك أشارت نتائج هذا الفصل إلى وجود دور مباشر ل PBDEs في زيادة خطر مقاومة الأنسولين المرتبطة بالسمنة وتحديد أنواعها الأكثر ارتباطاً بزيادة المخاطر.

خلاصة بحثنا هذا أننا قد حددنا بعض المستقلبات الأيضية التي تفرق بين OIS و OIR وبينا دور ملوثات بيئية محددة في زيادة خطر مقاومة الأنسولين المرتبطة بالسمنة. ستقوم أبحاثنا المستقبلية بتأكيد هذه العلاقات في مجموعات مختلفة وكذلك التحقق من طريقة عملها من خلال دراسة دورها في اعتراض عمل الأنسولين في وجود وغياب وسطاء الالتهابات وعوامل أكسدة وملوثات بيئية مختلفة. وسيهدف العمل المستقبلي أيضًا الى دراسة دور المستقلبات الأيضية في زيادة خطر مقاومة الأنسولين في الأشخاص غير البدينين بهدف تقييم استخدامها كمؤشرات حيوية تشخيصية محتملة وأهداف علاجية ممكنة.

Chapter 9: Appendix

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